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THE
JOURNAL
OF
MEDICAL RESEARCH

EDITED BY
HAROLD C. ERNST, M.D.

VOLUME XVI.
(New Series, Vol. XI.)

MARCH TO JULY, 1907

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THE Journal of Medical Research.

(NEW SERIES, VOLUME XI.)

VOL. XVI.

MARCH, 1907.

No. 1.

THE PRESERVATION OF NEUTRALITY IN CULTURE MEDIA WITH THE AID OF PHOSPHATES.*

LAWRENCE J. HENDERSON AND H. B. WEBSTER.

(*From the Laboratory of Biological Chemistry of the Harvard Medical School.*)

In order to acidify di-potassium phosphate enough acid to convert it into mono-potassium phosphate, that is to say one molecule of a monobasic acid, must be added to its solutions before their reaction becomes barely acid; and of course to solutions containing salts like acid potassium phosphate an equivalent amount of alkali must be added to obtain a slight alkaline reaction. These facts suggest that such substances or mixtures of them may be of use to obtain neutral culture media and to preserve the neutrality of such media during the growth of acid or alkali forming organisms upon them. This possibility is strengthened by the fact that phosphates are an important constituent of living protoplasm, where they must serve to neutralize acid or alkali produced by the metabolism of the cell or in any way introduced into it, as elsewhere pointed out by one of us.† Thus the use of phosphates for this purpose, closely simulating the conditions within the cell, seems a simple and natural device.

Over substances like calcium carbonate the phosphates have the important advantage theoretically, in neutralizing acids, that the chemical change occurs in solution and is instantaneous and complete throughout all portions of the solution; however, the actual advantages of the single phase reaction in this case can be tested only by experiment.

* Received for publication Nov. 22, 1906.

† Equilibrium in Solutions of Phosphates by Lawrence J. Henderson, *American Journal of Physiology*, XV., 257-271, 1906.

Accordingly the following experiments were carried out to test the usefulness of phosphates to hasten and increase the growth of *B. acidilactici*.

First Series.—Five hundred cubic centimeters of whey, freshly prepared, was inoculated with a pure culture of *B. acidilactici* suspended in one hundred cubic centimeters of water; three hundred cubic centimeters of the resulting liquid was then mixed with fifty cubic centimeters of a five per cent solution of Na_2HPO_4 and for control experiments the remaining three hundred cubic centimeters were diluted with fifty cubic centimeters of five per cent NaCl . After thoroughly mixing the preparations they were introduced in twenty-five cubic centimeter portions into test-tubes and placed in a thermostat at 40°C . The rapidity of growth of the organisms was estimated by titration from time to time of the acidity of the tubes, using $\frac{2n}{3}$ NaOH and as indicator phenolphthalein. The results of these titrations are collected in the following table:

TABLE I.

Solution to which Phosphate was added.			Solution to which no Phosphate was added.		
Time of Incu- bation.	Total Acidity.	Increase of Acidity.	Time of Incu- bation.	Total Acidity.	Increase of Acidity.
0	1.39n	0	0	1.36n	0
4 hours.....	3.28	1.89n	4 hours	2.32	0.96n
4 "	3.96	1.57	4 "	2.03	0.67
20 "	11.1	9.73	20 "	5.06	4.70
21 "	10.7	9.31	21 "	5.06	4.70
45 "	15.5	14.1	45 "	8.09	7.73
70 "	18.1	17.7	70 "	17.2	15.8
70 "	18.6	17.2	70 "	11.2	9.8
			70 "	8.43	7.07

The slight variation in the results may be due to slight settling of the bacteria during the measuring out of the proportions of the original solutions. Thus one portion might be started with rather more bacteria in it than others. The solution was separated into measured portions before incubation to avoid the error due to evaporation and concentration of the solution.

Clearly the production of acid is uniformly and very considerably greater in those tubes which contain phosphates, a result which in such a large number of single experiments cannot be ascribed to chance.

Second Series. — Four hundred cubic centimeters of five per cent glucose solution, fifty cubic centimeters of proteid free blood serum previously inoculated with *B. acidi lactici* and fifty cubic centimeters five per cent Na_2HPO_4 solution and a small quantity of asparagin were mixed. A similar solution for control was prepared using NaCl instead of Na_2HPO_4 ; twenty-five cubic centimeter portions of these preparations were introduced into test-tubes and placed in the thermostat at 40°C . The original glucose content of the two solutions was determined by titration with Pavy's solution. After three days the glucose content of the various tubes in which the organism had been growing was estimated. The results of these determinations are collected in the following table:

TABLE II.

Solution containing Phosphate.	Solution containing Sodium Chloride.
Initial Glucose Content, 0.57 %.	Initial Glucose Content, 0.62 %.
Portions examined after 3 days.	Portions examined after 3 days.
1st portion0.09 % glucose.	1st portion.....0.36 %
2d " trace only.	2d "0.37 %
3d "0.05 % glucose.	3d "0.36 %
*4th "0.37 % "	4th "0.35 %
5th "0.12 % "	5th "0.49 %
*6th "0.37 % "	6th "0.45 %
*7th "0.32 % "	7th "0.48 %
	8th "0.39 %
	9th "0.47 %
Mean.....0.19 %	Mean0.41 %

* These tubes showed but slight turbidity. For some reason the bacteria did not grow as profusely in them as in the others.

Even more striking than in the first series of experiments is the favorable influence of phosphates upon the growth of *B. acidi lactici* in this second series. Here again the results are on the whole surprisingly uniform, and it is not possible reasonably to explain them by chance coincidences.

The presence of phosphates in these experiments has greatly favored the growth of the acid-producing organism, as was expected from theoretical considerations; this favoring influence may reasonably be explained by the ability of the phosphate to neutralize the acid formed, though that explanation has not been conclusively established by the experiments.

In conclusion it may be pointed out that the neutrality of mixtures of mono- and di-phosphates within wide ranges of variation of alkali and acid content may be utilized readily to prepare neutral culture media without titration. If a

culture medium is found to be slightly acid or alkaline it is only necessary to add a few cubic centimeters of a moderately concentrated solution of alkaline or acid phosphate, as the case may be, and the reaction will be neutral.

It is hoped that this investigation may be of use in indicating a rational method of favoring the growth of acid-producing organisms and in providing a quick and easy means of obtaining neutral culture media.

THE ELASTIC TISSUE OF CARCINOMATA.*

GUTHRIE MCCONNELL, M.D.

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Although a number of investigators have examined into the formation of elastic tissue in tumors, yet the question as to whether or not new fibers are present still remains unsettled.

The following cases were examined primarily in regard to the above condition, but another point was considered. That one was in reference to the possibility of there being some relationship between the development of carcinomata, particularly of the skin, and changes taking place in the elastic tissue, either in quantity or quality, in advanced life.

In the skin of adults well on in life there is what appears to be an increase in the amount of elastic tissue. That this is merely apparent and not actual is agreed upon by most observers. The general opinion is that there is an atrophy of the connective tissue with a thinning of the skin and a crowding together of the elastic fibers.

Schmidt¹ believes "that the elastic degeneration is the main element in the senile changes of the skin, from the formation of the first elastic fragmentation to the transformation of the cutis into a structureless mass." He also believes that the apparent increase in amount of elastic tissue is due to a diminution in volume of the skin and a flattening of the papillary bodies.

Reizenstein² confirms Schmidt's results in general. He, however, found the degenerative changes in young people of twenty-six and twenty-eight.

Unna³ believes that the increase of elastic fibers in the skin is not due to new formation, but is the simple result of an irregular thickening of the fibers. He also claims that there is a degeneration of both the elastic and the collagenous bundles.

Orbant⁴ examined the skin from the bodies of twenty old people and found it much thinned with also marked changes

*Received for publication Nov. 25, 1906.

of the elastic tissue in the sub-epithelial layer. The fibers being thickened, irregularly arranged and showing a "colloid" degeneration.

Borrman⁶ in discussing the development of skin carcinomata speaks of a swelling and degeneration of the elastic fibers as one of the preliminary processes.

The thought arose as to whether on account of the degeneration of the elastic tissue there was not a loss of the restraining power that normally settles the point at which proliferation of cells should cease. That instead of there being a new formation of elastic tissue in tumors that there is a diminution, with a decrease in its power of keeping the general structures in a tonic condition. The point to be proven would of course have to be that in those people suffering from malignant disease there was a greater degeneration of elastic tissue than commonly occurs.

Sixty-five specimens of new growths, carcinomata, were examined; they were divided as follows:

- 36 Epitheliomata.
- 13 Rodent ulcers.
- 13 Carcinomata.
- 3 Malignant adenomata.

The method of staining was the acid orcein, followed by Unna's polychrome methylene blue. The latter being used for the purpose of revealing the presence of elacin, the form of degeneration in which the elastic tissue loses its affinity for the acid stain but takes up the basic.

The fixation in all cases was in Zenker's fluid, followed by imbedding in celloidin.

SQUAMOUS EPITHELIOMATA.

Of these there were thirty-two primary growths, three secondary and one recurrent. Twenty-six were from the skin, two from the tongue, two from the cervix, three secondary in lymph nodes and one unknown.

The changes in all of these specimens were so similar, differing merely in degree, that the same description will do for all.

With the exception of the secondary growths all but one or two of the others were so prepared as to include the uninvolved skin or mucous membrane.

The elastic tissue in all of these showed a distinct fragmentation in the areas uninvolved by the tumor cells. The fibers in many places had broken into pieces which were considerably thicker than the normal ones remaining. In some areas there was a distinct granulation of the fibers, these granules still retaining their affinity for the acid orcein.

As the periphery of the advancing growth is approached the amount of elastic tissue is generally considerably increased up to the edge of the zone of round cell infiltration. It then as a rule abruptly diminishes.

In twelve of the specimens there is distinct evidence of the pushing ahead of the fibers by the advancing growth. This may be indicated by a thick layer of fibers following the outline of the growth or at the point of an advancing papillary ingrowth the elastic tissue may be massed. In one section the fibers at the apex of the papilla have broken into irregular fragments, while along the side fibers remain quite long and delicate.

Within the zone of round cell infiltration and in the peripheral zone of the neoplastic tissue there are elastic fibers present, but in less amount than in the unattacked tissue. With the exception of seven specimens the elastic tissue becomes very distinctly less as the interior of the growth is approached. In twelve instances there is an almost complete disappearance of the elastic fibers.

Two of the sections, one from the lip, the other from a very early epitheliomata of the back of the hand following a burn, showed a very interesting condition. In the lip specimen all the fibers of elastic tissue instead of staining distinctly with the acid orcein took up the polychrome blue to the extent of becoming a very definite purple. The other section from the hand shows many fibers that have not been at all influenced by the orcein but have been acted upon by the basic polychrome dye staining a very definite blue. This reaction occurs not only in the immediate neighborhood

of the growth but in what appears to be healthy tissue on either side. The changes are apparently a degeneration of the elastic tissue into the elacin of Unna.

The action of the growth upon the elastic tissue would appear to be mainly a mechanical one, but another factor may have some bearing. Intervening between the surface of the growth and the surrounding tissue is a more or less well developed zone of round cell infiltration. The increased amount of elastic tissue is found beyond the round cells in the uninvolved areas. It may be that the round cells exert some direct influence upon the elastic fibers that helps to bring about their degeneration.

The ages of the patients in those cases in which it could be ascertained varied from forty to seventy-three with two exceptions. One a girl of nineteen in which there was an extensive proliferation of epithelium in a scar of a large burn of the thigh. The other case was that of an epithelioma of the cervix in a woman of thirty-four. Omitting the above two the average age was fifty-five.

RODENT ULCER.

Thirteen specimens were examined.

In these the conditions were practically similar to what have been described in the epitheliomata. In all of them there was marked fragmentation of the elastic tissue away from the growth as well as in it. The amount of the elastic fibers decreased greatly the further away from the periphery, and in one specimen there were almost no fibers, notwithstanding the fact that there was an unusual amount of connective tissue surrounding the cell nests. In another specimen taken from deep in the tumor there was no elastic tissue present, not even in the small fibrous areas containing the capillary blood vessels. A third slide showed an unusual amount of elastic tissue in the skin and surrounding the cell nests, but even in this one there was less elastin in the central portion of the growth.

Five out of the thirteen specimens also showed distinct evidences of the mechanical crowding together of the elastic fibers.

The average age of ten of the cases was sixty-four, the oldest seventy-seven, the youngest forty-nine. Three were in females, seven in males.

CARCINOMATA.

Of the thirteen specimens examined five were from the breast, four primary and one recurrent. The five showed quite similar conditions. There was very little elastic tissue present, many areas being almost completely free. What was found occurred in fragments and fibers scattered here and there in the connective-tissue trabeculæ. In four of these were found rings of elastic tissue enclosing tumor cells.

The other specimens were from the foot, uterus, esophagus, stomach, three secondary growths from the axilla, and one whose source was unknown.

In the thirteen there was more or less marked fragmentation with a decrease of the elastic tissue toward the center of the growth.

MALIGNANT ADENOMATA.

In these three specimens there was also fragmentation of the elastic tissue, and in two cases there was an almost complete absence of the tissue.

ELASTIC TISSUE RINGS.

In fifteen of the specimens out of the sixty-five examined, there were found round or oval rings of elastic tissue enclosing masses of tumor cells in between which there were occasionally found small blood vessels.

The origin of these rings seems to vary somewhat according to the site of the growth. In the epitheliomata of the skin there were in several instances the picture of an advancing papilla of tumor cells pushing a felt-work of elastic fibers ahead. It would be evident that if such a process was cut transversely, there would be found a mass of tumor cells surrounded by a ring of more or less degenerated elastic tissue.

In tumors arising from glandular structures such as the

breast, the rings are probably the remains of ducts that have been occluded by the new cells. In one specimen quite a large duct was found that was surrounded by a thick ring of fragments of elastic fibers. One side of the opening was covered by apparently normal epithelium, while on the other there were several layers of definitely atypical and abnormal epithelium.

In some specimens the rings presented a distinctly corrugated appearance and on that account were suspected of being blood vessels that had become thrombosed. This possibility was verified by the findings in one instance. In it there was present a long oval ring within which were several tumor cells and also some blood cells. This mass of elastic tissue could be traced continuously till it developed into a well formed blood vessel.

The following references concerning elastic tissue in new growths were consulted:

Melnikow-Raswedenkow⁶ in their article come to the conclusion that there is no increase of the elastic tissue in tumors. Hanseman⁷ says that in general there is no increase in tumors, but mentions a case of splenic metastases in which the elastin was apparently much increased. Tsutomu Inoye⁸ examined twenty cases of carcinoma of the stomach and speaks of the mechanical action of the growth upon the elastic fibers. The only new formation that he mentions is that which he found taking place in the immediate neighborhood of the blood vessels.

Wrench⁹ concludes that "the small meshed intra-alveolar stroma of carcinoma, whether primary or secondary, is devoid of newly formed elastic tissue, except in the neighborhood of vessels or ducts." In examining four cases of primary carcinoma of the liver he concluded that "the stroma of primary carcinoma of the liver is of at least two varieties: (a) Small meshed stroma, delicate or dense (scirrhous) free of elastic tissue. (b) Large meshed stroma containing abundant elastic tissue and indicating a preëxisting cirrhosis.

Fischer¹⁰ in the examination of many tumors concluded that in all chronic neoplasms elastic fibers were formed, but

that in the rapidly growing forms, particularly in carcinoma and sarcoma, the elastic fibers are generally lacking.

White¹¹ out of his seventeen cases says that "In only one case, a carcinoma of the breast, does there seem to be sufficient ground for claiming an increase in amount or of new formation."

Williams¹² concludes from his findings in thirty-seven cases of carcinoma that: 1. When the stroma of a carcinoma is itself of new formation it is usually free from elastic fibers. 2. Newly formed elastic fibers may occur in the stroma, though rarely, and they are likely to be fine in quality and small in number. 3. The tumors in which newly formed elastic fibers occurred either contained a large amount of connective tissue stroma or the newly formed fibers were in connection with preëxisting elastic elements of the original parts.

Hamilton¹³ reports a case of fibro-sarcoma of the brain in which there was a large amount of elastic tissue and which she believed to be of new formation, arising from the walls of the blood vessels and occurring in their neighborhood.¹⁴ She also examined scirrhus carcinomata from the pancreas, mammary gland, and liver and concluded "that the tumors containing the largest numbers of elastic fibers are those which possess a stroma comparatively rich in connective tissue cells." She states that "Almost all of the scirrhus carcinomata from those organs which were examined for this purpose were found to contain large numbers of elastic fibers, far more than in any of the fibromata examined. The fibers in some cases seemed to come from preëxisting ones around the ducts or vessels, but were far more abundant than in normal tissue and passed from these regions to the surrounding stroma. This was especially true of the two cases of cirrhosis of the liver originating in the bile duct epithelium."

It would seem to me that, at least, in both the hepatic and pancreatic growths, the primary condition had been a cirrhosis, following which a malignant growth had developed. In such a case the formation of the elastic fibers would have preceded the malignant change.

In a report of a transmitted mouse tumor Bashford¹⁵ in his description makes the statement that "there was no elastic tissue present." Such a case would seem to prove quite conclusively that the new formation of elastic fibers was by no means an essential part of the growth.

Tyzzar¹⁶ reports a case of primary carcinoma of the lung in a mouse, in which growth he says that the elastic tissue was present in larger amount than in the normal lung.

Zieler¹⁷ after a very exhaustive investigation and also a review of the work of others comes to the following conclusions:

1. Elastic fibers are almost constantly found in greater or less amount between the cell masses of skin carcinomata.
2. New formation of elastic fibers in the stroma of skin carcinomata cannot as a rule be demonstrated, but it is certainly occasionally observed in very slowly growing carcinomata.
3. Degenerative changes were no more frequent or marked in the elastic fibers in the involved region than in those of the neighboring skin, and never if they were lacking in the latter region.

Scheel¹⁸ in speaking of mammary carcinomata says that new formation of elastic tissue is not unusual, particularly in the scirrhous forms. It occurs especially in the walls of the ducts, but also in the vessels and interstitial tissue. Also that carcinoma in other parts can show an increase in the elastic tissue, but that in the majority of instances such is not the case.

With the one exception of the mouse tumor mentioned above it would seem that an examination of the literature shows that the formation of elastic tissue in malignant growths is very questionable. Those cases in which it has been found in an apparently increased amount are ones in which the possibility of a primary cirrhosis cannot be ruled out.

In none of the sixty-five specimens examined were there any indications that would lead one to suspect that there had been any new formation of elastic tissue. In all there was

a distinct diminution of the elastin the further away the area was from the periphery of the growth.

In all the cases there could be found very fine branching fibers, as a rule in the uninvolved areas. In a few instances such branchings were present in the connective tissue between the masses of tumor cells. They generally appeared as the splitting off of a small fiber from a larger.

Many of the specimens also showed quite plainly the mechanical effect of the extension of the growth. At its periphery, particularly well shown in the epitheliomata, could be found strata of elastic tissue fibers that had been crowded together. They would follow the contour of the growth and when the advancing process was a pointed one the fibers would be seen in a broken condition at the apex.

The ages of the patients from whom these specimens were taken varied in thirty-five instances from nineteen to seventy-seven. In the younger ones the changes found were those supposed to be characteristic of senile atrophy.

In six of the thirteen cases of rodent ulcer the duration of the condition was ascertained. The most recent was of five years standing, the oldest twenty-four. No definite relationship between the duration and the amount of elastic tissue could be shown. In the three-year case there was more elastin than in any of the others, while in one of fourteen years there was a large amount of connective tissue but only a very few small fragments of elastin.

Although the degeneration of the elastic tissue may have a bearing upon the formation of new growths nothing has been found in the above cases that allows of definite conclusions being drawn. The impression gained is that in the region of the growth itself most of the changes are mechanical, but fragmentation of the elastic fibers was found in what was otherwise apparently normal tissue.

The conclusions that seem warranted are :

1. That the formation of new elastic tissue does not commonly occur in new growths.
2. That there is a distinct degeneration of elastic tissue,

with in some cases the formation of elacin in malignant growths.

3. That the degeneration of elastic tissue in old age is one of the preliminary processes.

4. That the majority of cases in which there has been new formation of elastic tissue are those in which there may have been a preceding cirrhosis.

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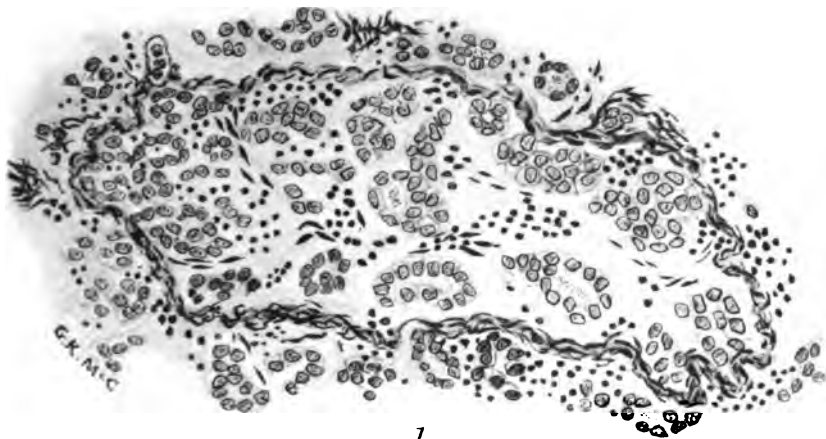
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DESCRIPTION OF PLATE.

FIG. 1. — Probable blood vessel obstructed by carcinomatous tissue, elastic tissue present as a tortuous ring. Adenoma malignum of stomach. $\times 300$.

FIG. 2. — Acinus showing beginning carcinomatous change. Elastic tissue in wall. Carcinoma of mammary gland. $\times 115$.

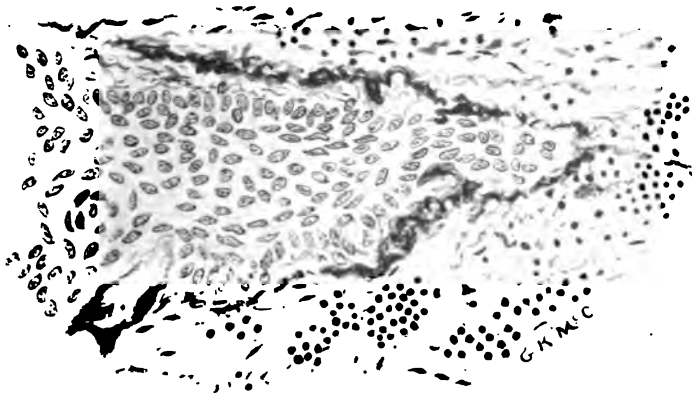
FIG. 3. — Advancing papillary process in an epithelioma of the skin shoving elastic tissue before it. $\times 300$.



1



2



3

ABSORPTION FROM THE PERITONEAL CAVITY.*

PART VI.

B. H. BUXTON.

(From the Department of Experimental Pathology, Loomis Laboratory, Cornell University Medical College, New York.)

This paper deals with a continuation of earlier studies,¹ more particularly of Part II., in which absorption of typhoid bacilli from the peritoneal cavity, and the distribution of the bacilli in the blood and organs, were considered.

In the present work an attempt has been made to determine the number of typhoid bacilli present in the blood of a rabbit at varying intervals after injection into the peritoneal cavity, the animal being sacrificed after four days, and the bacilli still remaining alive in the peritoneum and organs estimated.

Methods. — Normal rabbits of about five-pound weight were used, the dose being one-half of an agar culture of typhoid bacilli, approximately four thousand million bacilli. At various intervals after injection one cubic centimeter of blood was drawn from the ear, the drops of blood being allowed to fall into one cubic centimeter of sterilized ox bile; twenty-two drops are approximately one cubic centimeter. The ox bile prevents coagulation of the blood, and not only has no bactericidal properties of itself, but inhibits the bactericidal action of the drawn blood. The mixture of bile and blood is plated out in agar, and the number of bacilli per cubic centimeter of blood estimated from the colonies.

In previous experiments (Part II.) it was found that an injection of half an agar culture of the strain of typhoid used was not often fatal, but in the present series with the same strain the mortality has been much greater. No doubt this increased mortality is principally due to the successive bleedings just after inoculation, but I have also been led to the conclusion that the larger five-pound rabbits used for

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these experiments are somewhat more susceptible to the immediate effects of peritoneal infection than the smaller three to four pound rabbits used previously. There appears to be a critical time, about two hours after inoculation, at which the rabbit is very likely to die from the toxic action of the bacilli introduced. If this critical period is passed, the animal is likely to recover, although it may die in about twenty-four hours. In the latter case death is probably due to multiplication of the bacilli, and cumulative toxic action, although a marked increase in the number of bacilli cannot always be demonstrated. It was shown previously that after intraperitoneal injection, the bacilli very rapidly reach the blood in immense numbers, and then rapidly disappear from it, although the process was not demonstrated in any individual animal, as has been done in the experiments detailed in the accompanying Table I.

TABLE I.

Estimated number of bacilli per cubic centimeter of blood at varying intervals after injection of approximately four thousand millions of typhoid bacilli into the peritoneal cavity. Results with five rabbits which survived the inoculations.

Time.	Rabbits.					Approximate Averages.
	No. 1.	No. 2.	No. 3.	No. 4.	No. 5.	
5 minutes..	75,000	15,000	2,000	250	15,000	20,000
30 "	10,000	3,000	4,000	100,000	40,000	30,000
1 hour	500	—	100	400	—	350
2 hours	250	20	120	170	1,200	300
4 "	120	75	100	70	400	150
6 "	60	60	25	10	200	70
1 day	20	?	25	?	5	?20
2 days	0	?	0	?	?5	?
3 "	?30	?	?0	?	?50	?
"	15	20	0	0	0	7
	Sick on second day.	Suffered little at first. Sick on second day.	Very sick in two hours. Lost much weight.	Did not suffer at all.	Suffered little.	

Table I. confirms the previous observations that the bacilli very rapidly reach the circulation, from which they shortly disappear again to a great extent. The remarkable difference between the numbers in the circulation at thirty minutes and one hour is worthy of note. After the first hour the decrease is more gradual. The figures for one, two, and three days are not very reliable, since it has been found impossible at these periods to avoid a considerable degree of contamination, but the figures for four days are approximately correct since the blood was then taken from the carotid. When the ear is first shaved and washed it can be sufficiently sterilized to reduce contamination by skin cocci to a negligible quantity, but it appears that the blood clots which are unavoidably left on the ear over night permit of multiplication of skin cocci to such an extent that the ear cannot again be properly sterilized, and the colonies of contamination may so largely exceed those of typhoid bacilli that it is often difficult to determine the actual numbers of the latter even approximately.

Since the numbers of bacilli in the blood vary so much from one animal to another, the averages given in the last column of the table convey little meaning except in a very general way. The higher average in thirty minutes over five minutes is mainly due to Rabbit No. 4, and is completely reversed in Table II., which gives the results with rabbits which succumbed, but both tables show the decided drop in the number of bacilli between thirty minutes and one hour. Only in two instances in Table II. are there considerable numbers of bacilli in the blood at one hour. The other four rabbits show approximately the same numbers at one hour as those which survived.

TABLE II.

Estimated number of bacilli per cubic centimeter of blood at varying intervals after injection of approximately four thousand millions of typhoid bacilli into the peritoneal cavity. Results with six rabbits which succumbed to the inoculations.

Time.	Rabbits.						Approximate Averages.
	No. 6.	No. 7.	No. 8.	No. 9.	No. 10.	No. 11.	
5 minutes	5,000	75,000	100,000	200,000	50,000	100,000	100,000
30 "	1,500	10,000	?3,500	40,000	30,000	?1,000	20,000
1 hour ...	150	500	1,000	25,000	5,000	200	6,000
2 hours ..	100	600	†	†	2,500	†	1,000
4 " ..	30	250	2,000	?750
6 " ..	100	†	5,000	?
1 day....	†	15,000	?
	† 9 hours.	† 5 hours.	† 1½ hour.	† 2 hours.	Dying in 24 hours. Killed.	† 1½ hour.	

† Died.

Observations on Table II.

Rabbits 6 and 7 died respectively in nine and five hours. Death at these periods has not been observed with animals kept quiet after inoculation, and was probably accelerated by the rough handling incidental to the bleeding. As already remarked, two hours and twenty-four hours are approximately the usual critical periods at which a rabbit may die. Rabbit 10 shows a much larger number of bacilli in the blood at one hour and later than any of the others. This may be due to the fact that a different and more virulent strain of typhoid was used for the inoculation.

Distribution of the bacilli.—After four days the rabbits which survived were bled from the carotid and the relative numbers of bacilli still alive in the peritoneal cavity and organs were estimated according to the methods described in Part II.

Table III. shows the results obtained. The autopsies on these rabbits have always shown the spleen to be considerably enlarged, and the omentum thickened and covered with pus spots. Otherwise gross lesions have not been obvious, although the marrow may show signs of unusual activity.

TABLE III.

Colonies of typhoid bacilli on agar plates made from the various organs. The spleen is taken as a unit, and pieces of the other organs of approximately the same size, each piece being rubbed down with fifteen cubic centimeters of salt solution, and one cubic centimeter plated out.

Rabbit No.	Estimated bacilli alive in peritoneal cavity.	Liver.	Spleen.	Lung.	Marrow. One-fifteenth of one thigh bone.	Kidney.	Lymph nodes. Estimated bacilli in upper anterior mediastinal.	Blood, 1 cc.	Bactericidal power of serum.
1.	4,000	4	45	3	15	0	3,000	15	> Normal.
2.	0	1	60	0	3	0	100,000	20	> Normal.
3.	150,000	20	15	10	0	75	6,500	0	> Normal.
4.	500	1	10	65	2	0	8,000	0	> Normal.
5.	0	5	45	5	8	0	4,000,000	0	> Normal.
Approximate averages.	6	35	16	5 ex. ∞	71	7	

Table III. shows that the relative numbers of bacilli in the spleen are considerably higher than in the liver. This corresponds with previous observations at sixteen, twenty-four, and forty-eight hours; reversing the relations of these two organs as compared with the first few hours after inoculation, during which the numbers in the liver usually greatly exceed those in the spleen. The number of bacilli in the upper anterior mediastinal lymph nodes, through which, as was previously shown (Part III.) the bacilli pass on their way to the circulation, is still very considerable.

It seems that the bacilli tend to collect in the lymphopoietic organs, where they may remain alive for a considerable time. Our sections (Part III.) show that in an hour or two after injection indigestible particles and bacteria in the lymph nodes are practically all contained in the phagocytes, principally macrophages. It would appear probable, therefore, that the phagocytes must digest bacteria very slowly.

Table IV. gives the relative numbers of bacilli in the organs of five rabbits which succumbed. Rabbit 6 died late in the evening and was not tested.

The organs and peritoneal cavities of these five rabbits contain large numbers of living bacilli, although a rough calculation shows that only a small proportion of the entire dose inoculated can be accounted for. It seems probable that immense numbers of the bacilli injected have been rapidly destroyed, thus accounting for the early deaths from intoxication. This statement applies to Rabbit 10 (twenty-four hours) so far as the organs are concerned, but in the peritoneal cavity itself there has been an enormous increase of the bacilli. The significance of these findings will be discussed in the next section (VII.) of this work.

TABLE IV.

Colonies of typhoid bacilli on agar plates made from the various organs. Five rabbits which succumbed to the inoculations.

Rabbit No.	Estimated bacilli alive in peritoneal cavity.	Liver.	Spleen.	Lung.	Marrow. One-fifteenth of one thigh bone.	Kidney.	Lymph nodes. Estimated bacilli in upper anterior mediastinal.	Died in
7....	35,000,000	4,000	500	600	1,500	5	30,000,000	5 hr.
8....	120,000,000	4,000	3,000	400	4,000	20	12,000,000	1½ hr.
9....	120,000,000	7,500	3,000	750	3,500	150	35,000,000	2 hrs.
10 ..	28,000,000,000	600	10,000	5,000	3,500	70	50,000,000	Dying 24 hrs.
11....	20,000,000	1,500	3,500	350	750	8	4,000,000	1½ hr.

Bactericidal power of the serum.—Attention has previously² been drawn to the fact that after one or two injections of living typhoid bacilli the serum becomes more bactericidal than the serum of a normal rabbit, but on still further immunizing the animal, its serum rapidly loses in bactericidal power, and after a time is no longer bactericidal at all. It has been found with this series of rabbits that in four days after the single injection the serum is distinctly more bactericidal than normal. Table V. gives the averages of tests of the five rabbits killed four days after the inoculation. The fresh serum of a normal rabbit was tested in each case against that of the inoculated one.

TABLE V.

Bactericidal power of fresh serum four days after intraperitoneal injection of typhoid bacilli. In each tube two cubic centimeters of diluted serum, two drops of broth, and four drops of emulsion of typhoid bacilli. After five hours five drops of the fluid are plated out in agar and the colonies counted next day. Averages of five tests.

	2 cc. of serum dilution.	Colonies of typhoid bacilli from 5 drops of fluid in 5 hours. Averages of 5 tests.		Condition of fluid in 24 hours.				Agglutination value of 4 days serum.
		Normal serum.	Serum 4 days after inoculation.	Normal serum. 5 tests.		Serum 4 days after inoculation. 5 tests.		
				No. of tests sterile.	No. of tests showing growth.	No. of tests sterile.	No. of tests showing growth.	
1...	1-1	0	0	5	0	5	0	} About 1-400 in each case.
2...	1-2	45	0	5	0	5	0	
3...	1-5	1,300	2	3	2	5	0	
4...	1-10	6,000	75	2	3	4	1	
5...	1-20	20,000	720	0	5	3	2	

Controls. — Two cubic centimeters salt solution, two drops of broth, and four drops of emulsion of typhoid bacilli.

Average colonies in five drops of fluid:

At once, 4,000.

In five hours, 10-20,000.

In connection with the serum it may here be noticed that the bacteriolytic power of normal rabbit serum seems to vary inversely as the square of the dilution. In a series of earlier experiments it was found that fresh undiluted normal rabbit serum might be expected to kill about one million typhoid bacilli per cubic centimeter. If two million bacilli were added to one cubic centimeter of serum more than a million would be killed, but usually some bacilli would be left alive and multiply so that in twenty-four hours the serum would be swarming with bacilli. In a later series of experiments with diluted normal serum it has been observed that a dilution of one to ten acts in much the same way as undiluted serum treated with two million bacilli. That is to say, the bacilli are not often all killed. Now in this later series the number of bacilli added to each cubic centimeter of fluid has been about twenty thousand on an average, and $20,000 \times 10^2 = 2,000,000$.

One cannot speak definitely, but the results of a large

number of tests indicate very strongly that the bactericidal action of the serum varies inversely as the square of the dilution. Or, to put the case simply — One cubic centimeter of serum, diluted one to ten, kills, not one-tenth the number of bacilli which one cubic centimeter of undiluted serum will kill, but only one one-hundredth of that number.

The literature on the subject of absorption from the peritoneal cavity was largely dealt with in previous sections of this series of papers and need not be referred to again, but a recent work by Rodet and Delanoë³ calls for some comment. These authors discuss the question of experimental typhoid fever and maintain that there is a radical difference between intravenous and intraperitoneal injection. After intravenous injection of a fatal dose the bacilli are destroyed in great numbers and death ensues without any obvious secondary multiplication of the bacilli. In this case there is intoxication. After intraperitoneal injection the process is localized, and death is caused by local multiplication of the bacilli. The authors admit, however, that some bacilli may find their way into the circulation after intraperitoneal injection, but they do not regard this as a true systemic infection such as is caused by intravenous inoculation.

Their assumption that there is a radical difference in the results after each kind of inoculation does not seem to be justified in the light of the experiments detailed here. Both in the present and previous communications it has been clearly shown that injection into the peritoneal cavity induces an almost immediate systemic infection, at any rate if the dose is large, and Rodet and Delanoë used very large doses.

A later paper will deal with the results obtained after injection of comparatively small doses of bacilli, and a discussion of the bearing which these experiments have upon the question of peritonitis in the human subject will be reserved for the present.

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3. *Archives de médecine expérimentale*, xviii, 1906, 581.

ABSORPTION FROM THE PERITONEAL CAVITY.*

PART VII.—THE EFFECT OF MINIMAL DOSES OF BACTERIA
AND THE BEARING OF THE EXPERIMENTS UPON THE
QUESTION OF PERITONITIS.

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Medical College, New York.)*

In experiments described in previous sections of this study, injections into the peritoneal cavity of rabbits were made with typhoid bacilli in very large doses, about one-half of an agar culture being used for each inoculation. After such large doses it was found that the bacilli almost immediately reach the blood in immense numbers by way of the lymphatics of the diaphragm, which communicate with the lymphatic trunks of the anterior mediastinum.

The work has been extended in the direction of minimizing the doses given, and of determining if the results obtained with typhoid bacilli apply equally well to other kinds of bacteria. It has in fact been found possible to recover the bacteria from the blood, anterior mediastinal lymph nodes and liver within ten to fifteen minutes after intraperitoneal inoculation of doses so low as approximately fifty thousand to a hundred thousand living bacteria. Organs other than the liver contain so few bacteria after these minimal doses that they have been left out of account in making up the tables. It may be taken for granted that the liver is the only internal organ in which microorganisms are likely to be detected under these conditions. On the other hand, it is important to include the anterior mediastinal lymph nodes, since it is through the lymphatic system of the anterior mediastinum that bacteria find their way from the peritoneum to the blood current.

*Received for publication Jan. 30, 1907.

METHODS.

1. The dose. — A twenty-hour agar culture of the micro-organism to be used for injection is suspended in salt solution and diluted as required, five cubic centimeters of diluted suspension being always used for each inoculation. That portion of the culture dilution not used for inoculation is further diluted in test-tubes and agar plates made from the various tubes. In this way the number of bacteria used for inoculation can be approximately calculated. Details of the methods may be found in Part II. of this work.

2. The blood. — Immediately after inoculation the rabbit is etherized and bled from the carotid, five cubic centimeters of the blood being run into five cubic centimeters of ox bile. The bile prevents coagulation of the blood, and plates made from the mixture afford an estimate of the number of bacteria per cubic centimeter of the blood. Not more than ten minutes elapses between the inoculation and taking the sample of blood. It has been found in previous experiments with typhoid bacilli that the blood contains more bacilli during the first half hour after intraperitoneal inoculation than at any time thereafter, the maximum number being usually found between five and fifteen minutes after injection. The total number of bacteria in the blood is roughly estimated according to the size of the animal, rabbits of about three pounds weight being used. There are approximately one hundred cubic centimeters of blood in each animal of this size.

3. The peritoneal cavity is washed out and the number of bacteria remaining alive and free in the cavity is estimated according to methods described in Part II.

4. The upper anterior mediastinal lymph nodes are picked out and rubbed down with ten cubic centimeters of salt solution; one cubic centimeter being plated out. The total number of bacteria in the nodes is estimated from the colonies on the plates.

5. The liver is taken out and about two grams rubbed down in ten cubic centimeters of salt solution, of which one

cubic centimeter is plated out. This amount of the emulsion represents approximately the three hundredth part of the liver for one plate. The colonies on the plate are therefore multiplied by three hundred in order to estimate the total number of bacteria in the liver as given in the tables:

TABLE I.
Seven rabbits killed immediately after intraperitoneal injection of Bacillus typhorus.

	Dose.		Blood.		Estimated.			Number of Bacilli Accounted for.	Percentage Accounted for.
	Fraction of Culture.	Estimated Number of Bacilli.	Per Cubic Centimeter.	Estimated Total in Blood.	Bacilli Alive and Free in Peritoneal Cavity.	Bacilli in Upper Anterior Mediastinal Lymph Nodes.	Bacilli in Liver.		
1	1/2,000	5,000,000	Numerous.	?	3,000,000	50,000	50,000	?	
2	1/2,000	4,500,000	350	35,000	2,200,000	20,000	100,000	2,400,000	50
3	1/10,000	1,200,000	85	8,500	500,000	12,000	10,000	530,000	44
4	1/20,000	600,000	250	25,000	350,000	600	8,500	385,000	64
5	1/20,000	450,000	100	10,000	250,000	10,000	10,000	280,000	62
6	1/100,000	120,000	8	900	75,000	1,000	750	78,000	65
7	1/200,000	60,000	2	270	?	350	600	?	

TABLE II.
Six rabbits killed immediately after intraperitoneal injection of Bacillus coli communis.

	Dose.		Blood.		Estimated.			Number of Bacilli Accounted for.	Percentage Accounted for.
	Fraction of Culture.	Estimated Number of Bacilli.	Per Cubic Centimeter.	Estimated Total in Blood.	Bacilli Alive and Free in Peritoneal Cavity.	Bacilli in Upper Anterior Mediastinal Lymph Nodes.	Bacilli in Liver.		
1	1/20,000	1,000,000	100	10,000	560,000	600	0?	570,000	57
2	1/20,000	1,000,000	160	15,000	500,000	1,500	50,000	570,000	57
3	1/100,000	200,000	30	3,000	60,000	6,000	4,000	73,000	36
4	1/200,000	100,000	10	1,000	40,000	800	3,300	45,000	45
5	1/200,000	100,000	20	2,000	60,000	1,200	600	64,000	64
6	1/1,000,000	20,000	.5	50	12,000	100	0	12,150	61

TABLE III.
Five rabbits killed immediately after intraperitoneal injection of Staphylococcus pyogenes aureus.

	Dose.		Blood.		Estimated.			Number of Bacilli Accounted for.	Percentage Accounted for.
	Fraction of Culture.	Estimated Number of Bacilli.	Per cubic Centimeter.	Estimated Total in Blood.	Cocci Alive and Free in Peritoneal Cavity.	Cocci in Upper Mediastinal Lymph Nodes.	Cocci in Liver.		
1	1/10,000	1,000,000	100	10,000	400,000	20,000	15,000	450,000	45
2	1/20,000	350,000	40	4,000	60,000	10,000	10,000	85,000	25
3	1/100,000	100,000	13	1,300	75,000	1,200	1,800	80,000	80
4	1/100,000	100,000	20	20	50,000	500	20	?	250
5	1/200,000	35,000	2	200	16,000	450	500	17,000	50

TABLE IV.
Six rabbits killed immediately after intraperitoneal injection of Bac. pyocyaneus and Bac. prodigiosus.

	Culture of Bacillus.	Dose.		Blood.		Estimated.			Number of Bacilli Accounted for.	Percentage for.
		Fraction of Culture.	Estimated Number of Bacilli.	Per Cubic Centimeter.	Estimated Total in Blood.	Bacilli Alive and Free in Peritoneal Cavity.	Bacilli in Upper Anterior Mediastinal Lymph Nodes.	Bacilli in Liver.		
1....	Pyocyaneus.	1/20,000	2,000,000	80	8,000	1,000,000	25,000	15,000	1,050,000	52½
2....	"	1/200,000	200,000	50	5,000	150,000	3,500	4,500	165,000	82½
3....	Prodigiosus.	1/20,000	1,500,000	110	11,000	750,000	15,000	30,000	810,000	54
4....	"	1/50,000	600,000	2	200	200,000	7120	0?	?	?
5....	"	1/200,000	150,000	12	1,200	100,000	400	1,800	105,000	70
6....	"	1/500,000	60,000	2	200	15,000	300	500	16,000	27?

Experiments with anthrax and cholera were unsuccessful.

The tables show very clearly that some of the bacteria injected into the peritoneal cavity, even in minimal amounts, almost immediately find their way into the circulation. Roughly speaking, about fifty to sixty per cent of the total number injected can generally be demonstrated as remaining alive and free in the peritoneal cavity, and about two to five per cent in the blood, anterior mediastinal lymph nodes and liver. Owing to unavoidable imperfections in the methods employed, this statement can only be represented as affording a measure of the "order" of the systemic invasion. No approach to actual correctness of the individual figures can be claimed. Of the forty per cent of the bacteria injected which cannot be accounted for, some are no doubt distributed among the other organs in small amounts, some may already have been killed, but it seems probable that the greater portion are already fixed upon the omentum; partly sticking to its surface, and partly contained in the phagocytes (macrophages) which are normally present in the peritoneal cavity, and become massed upon the omentum immediately after the introduction of foreign matter. Microscopical examination of the omentum confirms this view, and there seems to be little doubt that the omentum plays an important part in the disposition of bacteria introduced into the peritoneal cavity by intercepting the rush to the diaphragm, but this aspect of the question will not be discussed further at present (see Parts IV. and V. of this series of articles).

The bacteria made use of in these experiments are such as can be easily demonstrated by plating methods. The streptococcus and pneumococcus were not tested, but Wallgren found them in the blood in ten to sixty minutes after intraperitoneal injection of streptococci, and Jensen demonstrated the same with pneumococci. It may therefore be taken for granted that systemic invasion after intraperitoneal injection into rabbits is a universal rule for all bacteria, and the same may probably be said of any inert matter in fine suspension.

THE BEARING OF THE EXPERIMENTS UPON THE QUESTION OF PERITONITIS.

In addition to these experiments with rabbits some have also been made upon guinea-pigs with similar results, but it would be unnecessary to give them in detail. It may also be mentioned that Muscatello and others have shown that carmine and various inert particles injected intraperitoneally into dogs reach the anterior mediastinal lymph nodes very rapidly. It is not often safe to argue from the experimental animal to the human subject, but it appears probable that rapid absorption of bacteria or particles from the peritoneal cavity through the lymph channels of the diaphragm is of universal occurrence in mammals. Rajewski indeed has shown that the lymphatics of the excised human diaphragm will absorb particles of Indian ink and carmine with great rapidity. Allowing, therefore, that the observation is of general occurrence the experiments detailed here seem to have some bearing on the question of peritonitis and its operative treatment.

Having had no clinical experience I can speak with no authority on the subject, but have merely attempted to select from the literature the opinions of a few of the more prominent writers, and discuss their views in connection with these experiments on rabbits. The clinician will be able to fill in the gaps for himself.

THE BACTERIOLOGY OF PERITONITIS. — Veillon and Zuber and more recently Ghon and Much very constantly found certain bacteria of a strictly anaërobic nature in foul smelling abscesses of the peritoneum, and concluded that these frequently take a prominent part in the causation of peritonitis, but most investigators, Hewetson, Dudgeon and Sargent for example, have been quite unable to confirm these observations, and do not consider that anaërobic organisms are of any significance in peritonitis. In the opinion of these authors *B. coli communis*, *B. pyocyaneus*, streptococci and pneumococci are the chief agents in severe and fatal cases.

Gonococci are said to be comparatively harmless, though fatal cases have been known to occur. Cushing concludes that "Gonococcic peritonitis, although it may occur, more commonly in females, runs a benign course and seldom comes to the surgeon in its acute state." Infection by

the staphylococcus pyogenes aureus is uncommon except, according to Flexner, in cases due to wound infection, exogenous peritonitis, as opposed to infection from the intestinal tract, endogenous peritonitis.

Dudgeon and Sargent lay great stress on the importance of the staphylococcus albus in peritonitis on account of its protective action. Of itself Staphylococcus pyogenes albus is innocuous, but when, as is frequently the case, it is associated with more virulent organisms, it actually serves as a protection by exciting leucocytosis. In the absence of the Staphylococcus pyogenes albus the prognosis according to these authors is less favorable. Of virulent organisms, the streptococcus and the pneumococcus are the most dangerous. There is little or no formation of pus, and seldom any effective localization. Dudgeon and Sargent question whether there is ever recovery from a pure streptococcic peritonitis. In the great majority of perforative cases *B. coli communis* is the chief infective agent, and with this organism there is ordinarily a more or less successful effort at localization with pus formation, and the peritonitis is not so liable to become generalized as with the streptococcus. Infection by *S. pyogenes aureus* and *B. pyocyaneus* is infrequent, but broadly speaking, *B. pyocyaneus* may be considered somewhat more, and *S. pyogenes aureus* somewhat less virulent than *B. coli communis*. Mixed infections naturally often occur, and the virulence of a bacillus or coccus varies within wide limits, so that the above statements must not be taken too literally.

TWO CONSIDERATIONS IN THE OPERATIVE TREATMENT OF PERITONITIS.

A. Posture: Clarke, 1898, realizing that absorption of fluids from the peritoneal cavity takes place very rapidly through the lymphatics of the diaphragm, recommended raising the pelvis after peritoneal operation with the object of assisting the natural drainage from the cavity. But this procedure has not been found beneficial, and the tendency now is to lower rather than to raise the pelvis. Murphy keeps his patients in a semi-sitting posture, at an angle of thirty-five to forty degrees, both before and for several days after operation. The semi-sitting Fowler position is to-day in frequent use.

B. Irrigation: There seems to be at present a decided reaction against the formerly universal practice of irrigating the peritoneal cavity as thoroughly as possible after laparotomy for inflammatory conditions. Symonds writes in regard to operation for peritonitis with perforation: "Since I have adopted the plan of free incision, dry mopping and

drainage, the results have greatly improved. In borderland cases operated upon on the fifth or sixth day I am certain that flushing will turn the scale to a fatal issue."

Murphy reported six cases of recovery after typhoid perforation, in none of which irrigation was practised. Murphy's view is that bacteria multiplying in the cavity destroy the epithelial lining of the peritoneum so that absorption of toxic products takes place. So long as the epithelium is intact there is little or no absorption. After irrigation the bacteria are likely to become generalized so that a larger surface of the peritoneum is liable to be denuded of epithelium. The semi-sitting position is also a help since absorption does not take place so readily from the lower parts of the abdominal cavity as from the upper, "and the patients are saved from the primary overwhelming dose of toxins which is so fatal." Dudgeon and Sargent also urge very strongly against irrigation as a rule. According to their views, with a streptococcus infection the generalization is so rapid that washing out is the best procedure, but it is doubtful if there is ever recovery from such an infection. Usually *B. coli communis* is the infective agent and this tends to become localized. Flushing is apt to generalize the infection and also washes out the phagocytes. "Pus in the cavity appears to the surgeon as something to be washed away," but it is precisely in the pus that there is protective action due to the innumerable phagocytes which it contains. Dry sponging is better than irrigation. Delbet removes the appendix and leaves the pus. The appendix is the laboratory, and when this is removed the supply of microorganisms is cut off, the phagocytes present in the pus making short work of the bacteria already present. Since abandoning irrigation he has had much better results.

Bond, in the opening paper of "A discussion on acute septic peritonitis," at the recent meeting of the British Medical Association in Toronto summarizes these views as follows: "It is in fact on the integrity of the endothelial and phagocytic defence that the safety of the patient depends; for if this breaks down, either from failure to react to the poison, or from loss of phagocytes by unwise irrigation, or by injury

to the endothelium by rough handling on the part of the surgeon, then the vascular channels of absorption are laid directly open, toxins enter the blood stream in overpowering doses and the patient succumbs from septic intoxication."

In the light of the experiments on rabbits the soundness of the argument against the irrigation and the probable benefits of the semi-sitting position will be at once recognized, but none of the authors mentioned seem to have grasped the main significance of the situation. Unquestionably the generalization of microorganisms in the cavity is highly injurious, but not so much because the peritoneal epithelium is destroyed or the phagocytes washed away, as because the generalization is liable almost immediately to allow of a systemic invasion with its attendant dangers. Murphy's dictum, "The primary overwhelming dose of toxins," is significant, but should be paraphrased as "The primary overwhelming dose of bacteria."

A remark of Dudgeon and Sargent's (p. 3) may also be quoted: "Whatever absorption cannot take place by the lymphatic channel must do so by means of the blood stream directly, and it is this which constitutes one of the greatest perils of peritonitis. Provided that the epithelium is uninjured, bacteria and other foreign substances will, within limits, be safely disposed of by the lymphatic route, but damage will at once permit absorption to take place by the vascular route." This view seems to me to be misleading. The bacteria are *not* safely disposed of by the lymphatic route even "within limits." On the contrary, very minute quantities of bacteria when set free in the peritoneal cavity are carried by the lymphatic route into the blood with extraordinary rapidity.

If we consider the case of a perforation with admission of *B. coli communis* into the peritoneal cavity there are probably at first comparatively few bacilli and an attempt, usually successful, at localization. Moreover, the omentum can probably intercept practically all of the bacteria provided they are diffused slowly throughout the cavity. But let the operation be performed and the cavity irrigated, and at once

the conditions are analogous to those obtaining in the experiments on rabbits. There may be immediate absorption through the diaphragm even from the first rush of the irrigating fluid, which as it passes over the infected areas picks up vast numbers of bacteria and carries them toward the diaphragm. Harte and Ashhurst collected three hundred and sixty-two cases of operation after typhoid perforation with a mortality of seventy-four per cent.

A portion of one of their tables may be given :

	Treatment.	Recovered.	Died.	Mortality Per Cent.
1	Drainage, no wiping or irrigation.	10	7	41
2	Wiping and drainage.	12	19	61
3	Irrigation and drainage.	46	130	74
4	Wiping, irrigation and drainage.	1	10	90

This table certainly seems to indicate that the less the treatment the more favorable the chances of recovery, although one must recognize the possibility that in some of the milder cases irrigation was not thought necessary. It is worthy of note, however, that where the usual practice has been carried out the percentage of mortality is exactly that of the whole number of cases; where more care has been exercised the mortality rises to ninety per cent, and with less care sinks to fifty per cent, the mean of these two being practically the same as for the entire number of cases. In this connection the following passage from Stimson may be quoted: "Closely related to this exaggeration (of operative interference) is that of the logical surgeon who . . . carries his knife to its ultimate conclusions, unmindful of the modifying conditions. . . . One of the most striking effects of this is seen in the ardor of the chase for germs. . . . One of the milder examples of this (ardor) which seems happily to be yielding to increasing experience is the

exaggerated washing and drainage of the abdominal cavity lately so prevalent."

Causes of death from the absorption. — In previous communications it has been mentioned that after inoculation of a large dose of living typhoid bacilli two hours is a critical period at which the rabbit is likely to die suddenly in convulsions or may become comatose and die a few hours later. If this early critical period is safely passed there is usually recovery, but the animal may die in about twenty-four hours. As a rule, though, there are frequent exceptions, a rabbit which dies in about two hours is found to have destroyed a large proportion of the bacilli injected, whereas the animal which dies in twenty-four hours is swarming with bacilli. Presumably in the first case the cause of death is the immediate toxic action of the disintegrated bacilli (endotoxins) directly upon some specially susceptible foci, such as nerve centers, whereas death after twenty-four hours is due to the general infection.

This view is supported by the fact that if a rabbit is inoculated intravenously with a small dose of typhoid bacilli, killed by heating to 60° C. for one hour, there is a critical period between one and two hours after injection. The critical period in this case comes on somewhat earlier than after intraperitoneal injection of living bacilli, but the symptoms are precisely similar in both instances. But the fact must again be emphasized that it is primarily the bacteria and not their toxins which are absorbed from the peritoneal cavity. On extracting from Dudgeon and Sargent's cases, I find that of deaths after operation and irrigation, twelve occurred within a few hours, while ten cases lived longer, dying after two to ten days. Probably the cases of death within a few hours correspond to the deaths among rabbits from intoxication, whereas the later deaths are due to a general infection, or more probably to generalized peritonitis as will be explained later, and correspond to the later critical period for rabbits. A number of significant instances may also be found among one hundred and twelve cases of operation for

typhoid perforation collected in detail by Finney. Is it not possible that many deaths after operation attributed to shock are in reality due to sudden systemic invasion by bacteria with rapid intoxication by endoscins?

In view of these observations upon the critical periods for rabbits some suggestions may be put forward as a working hypothesis by way of explanation for certain phenomena observed in peritonitis. Since the streptococcus and the colon bacillus are by far the most important etiological factors in peritonitis, remarks may be confined to these two organisms. Apart from any question of peritonitis the streptococcus very frequently causes systemic infection, whereas such an infection by *B. coli communis* is very uncommon and it is by no means well established that the colon bacillus can ever produce a true bacteremia in man.

Peritonitis due to streptococcus. Chiefly puerperal.—As has been mentioned, Dudgeon and Sargent consider that such infections are almost invariably fatal, though Dr. L. A. Stimson informs me that he is now not nearly so much concerned as formerly at the finding of streptococci in cases of peritonitis. It is, however, generally conceded that a streptococcic peritonitis is very dangerous, and is frequently followed by death. But it appears from a review of the literature that as a general rule death seldom follows within a few hours after operation. The patient usually succumbs in about twenty-four to forty-eight hours. There are relatively few deaths from operative "shock."

Peritonitis due to *B. coli communis*. Chiefly perforative.—On the other hand, after typhoid or other perforation *B. coli communis* is the chief infective agent, and after operation with irrigation of the cavity one cannot help being struck with the large proportion of deaths within a few hours after operation. One may almost say that death in such perforative cases, when it occurs after operation, is a matter of a very few hours or some days, with a gap in between, within which relatively few deaths occur.

Now it is just in the period, twenty-four to forty-eight hours, corresponding to this gap for *B. coli communis* that most of the streptococcic deaths seem to occur.

These conclusions have only been reached from a general survey of the literature, and are open to criticism by those who have had practical experience, but supposing it to be conceded that they are in the main correct, it seems to me that the following is a probable explanation of the phenomena. The streptococcus very rapidly generalizes and rapidly reaches the circulation whether there is irrigation of the cavity or not. On reaching the circulation it may find a favorable medium for growth either in the blood or at various foci in the internal organs. It is not immediately destroyed in large amounts, and therefore little or no liberation of toxin endotoxin takes place during the early period of the systemic invasion.

But the streptococcus rapidly multiplies, and in twenty-four to forty-eight hours has reached a point at which the toxins from disintegrating cocci are liberated in sufficient amount to cause death.

When *B. coli communis* the situation is different. At the moment of operation we may suppose the infection to be more or less localized, so that there has been no or only very gradual fusion of bacteria in the direction of the diaphragm, and up to this time few or no bacilli have reached the circulation. On irrigation of the peritoneal cavity during the operation there is a sudden rush of bacilli to the blood and organs, as we may infer from our experimental tests. But the blood and internal organs form an unfavorable environment for the bacilli, and large numbers are immediately killed off toxic products being liberated at various susceptible foci with consequent sudden death, the symptoms closely simulating those of shock, though indistinguishable from those of toxic poisoning. If the bacilli which have been thus introduced into the circulation are few in number or not particularly virulent, the patient survives the shock period, but the peritoneal infection being generalized

the bacilli multiply in the cavity and from there fresh bacilli are constantly being carried into the blood stream with cumulative toxic effect, and death after a few days. One might say that with streptococci death after operation is due to systemic infection, whereas with *B. coli communis* it may be due to rapid or gradual systemic invasion with consequent intoxication.

SUMMARY.

1. Bacteria injected into the peritoneal cavity of experimental animals even in minute doses reach the circulation in a few minutes.
2. This immediate absorption of bacteria takes place by the lymphatics of the diaphragm.
3. Assuming such immediate absorption to take place also in the human subject, the experiments have some bearing on the subject of operative peritonitis and its treatment.
4. To avoid a rush of bacteria to the diaphragm the Fowler position is indicated, and flushing of the peritoneal cavity contraindicated.
5. Rabbits after intraperitoneal injection of living typhoid bacilli frequently die from toxemia in about two hours. This indicates the probable cause of so many deaths from "shock" after operation for perforation with irrigation.
6. If a rabbit survives the "shock" period it may die in about twenty-four hours. This appears to correspond to the deaths after operations for perforation in which the fatal termination is reckoned in days instead of hours.
7. The above conclusions apply only to cases in which *B. coli communis* or allied species are the infective agents. Reasons have been given for supposing that streptococcus infections run a different course.

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THE PATHOLOGY OF THE BROWN-TAIL MOTH DERMATITIS.*

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The disagreeable dermatitis caused by the brown-tail moth has attracted much attention in the vicinity of Boston in recent years. At first of interest chiefly to dermatologists as a skin affection new to this region, this dermatitis has since become so common in moth-infested districts that it is now recognized by laymen as well as by physicians.

So much has been written concerning the brown-tail moth, *Porthesia* or *Liparis chrysorrhœa*, that it is unnecessary to review its life history and habits in this paper. Full information on these points, together with an account of the introduction of the moth into this country and its subsequent multiplication, is given in the report of Fernald and Kirkland.⁷

In June, 1901, Dr. J. C. White¹⁷ in a letter to the editor of the Boston Medical and Surgical Journal called attention to a peculiar type of dermatitis which he thought was undoubtedly due to the caterpillar of the brown-tail moth. The dermatitis, which was urticarial in character, usually occurred on the neck, although the face and hands were sometimes affected. All patients afflicted gave a history of the removal of a caterpillar from the parts affected just prior to the appearance of the eruption.

Soon after this Dr. E. R. Meek¹¹ likewise in a letter to the editor of the Boston Medical and Surgical Journal ascribed the dermatitis to the hairs of this caterpillar, since these elements are very brittle and easily detached.

Fernald and Kirkland, in the report already referred to, state that the irritation is produced only by certain short barbed hairs, which they term "nettling hairs," and by no others.

* Received for publication Dec. 4, 1906.

The irritating properties of species closely allied to the brown-tail moth have long been known to European entomologists. There is in the London Entomologist a series of notes recording the observations of a number of entomologists who have experienced irritation of the skin after handling specimens of these closely allied species.

In the year 1865 South,¹⁴ after collecting specimens of *Porthesia similis*, experienced intense itching, urticaria about the neck, and edema of the eyelids, but did not at that time ascribe the condition to the handling of the moths.

Rendall¹⁵ found that the cocoons as well as the larvæ may produce irritation.

Anderson¹ found that handling the imagines of *Liparis auriflua*, another species closely allied to the brown-tail, was followed by urticaria. He further stated that merely to walk, during a breeze, by certain hedges infested by this species is sufficient to produce the rash.

Swinton¹⁶ held the opinion that the hairs of the caterpillars are coated with a poisonous substance which exudes from the scarlet warts on the hinder segments.

The fact that cocoons, empty and exposed to the weather for months, are still capable of producing the rash, seemed to Cockerell² and others to militate strongly against the theory that the effects are caused otherwise than by the mechanical action of the hairs.

An interesting series of experimental inoculations is furnished by Carter.³ He inoculated his own skin with various species with the following results. — *Porthesia similis* produced redness, itching, pimples, and white vesicles on the skin. *Bombyx rubi* produced intense irritation, vesicles some of which became pustular, and edema of the eyelids; *Bombyx quercus*, pimples which became vesicular and afterwards dried up; and *Bombyx nuesta*, great irritation but no vesicles. Inoculation with *Arctia caia* and *Arctia villica* was followed by transient irritation. *Dasychoia pudibunda* caused red patches, and vesicles resembling chicken-pox. Other species were tried but proved to be innocuous.

Thresh¹⁶ calls attention to short hairs, 1/200 of an inch long, and barbed throughout their entire length, but thinks that the long hairs as well have nettling properties. He states that the long hairs give an acid reaction when they come in contact with litmus.

Perhaps the most notorious of the "stinging" larvæ is *Cnethocampa pityocampa*, the processionary caterpillar of Europe. Its poisonous properties were known to the ancients, as is shown by the fact that it is mentioned in the Cornelian law.¹⁰ Keller^{8, 9} investigated this species and found structures at the bases of the spines which he interpreted as poison glands.

There is in the Philippine Islands a moth, *Taragama igniflua*,¹² which resembles somewhat the brown-tail. Its nettling hairs were examined by the author and found to be almost identical with those of the latter species and capable of producing severe irritation. There were also long, needle-shaped, hollow spines, which were filled with fluid. As they were watched under the microscope the fluid rapidly evaporated leaving the spine empty. Although these spines were well adapted for penetration, it was not determined whether they were instrumental in the production of the dermatitis. The larvæ of the Io moth (*Automeris io*) also produce irritation of the skin when handled. There is an immediate sensation of pain similar in nature to that produced by the sting of a bee, but much milder in degree. There is some reddening of the skin, but the irritation is transient and soon disappears.

The larger portion of the work upon which the present paper is based was done several years ago when the moths first became prevalent in the suburbs to the north of Boston, but certain additional data have been obtained more recently. In investigating the nature of this peculiar skin eruption, the primary object was to determine by means of experimentation just how the lesions are produced. My observations confirm those of Fernald and Kirkland that the dermatitis is

produced by the peculiar, short, barbed spines or "nettling hairs" * of the caterpillar.

These hairs when rubbed upon the skin produce a dermatitis, but the other hairs of either the caterpillar or the moth produce no irritation. Furthermore, by serial sections of lesions produced experimentally, I have been able to demonstrate the nettling hairs imbedded in the skin. The most important result of this investigation is the proof that the action of the nettling hair upon the tissue is not a purely mechanical one, as the observations of Fernald and Kirkland and others tend to indicate, but that there is in addition an irritating substance. When this irritating substance is removed from the nettling hairs they are then practically innocuous and, when inoculated, act merely as foreign bodies in the tissue.

The nettling hairs are of the form of straight, tapering, needle-pointed shafts, barbed for their entire length after the manner of a certain form of African spearhead. They vary from .07 to .2 millimeter in length,—the average is about .1 millimeter or 1/250 of an inch,—and are not over .004 or .005 millimeter in thickness at the thicker extremity. They possess a thin chitinous wall from which project three rows of recurrent barbs, while the interior of the shaft consists of material which appears finely granular and stains with the ordinary anilin dyes after fixation in Zenker's fluid. When the nettling hairs are thoroughly dried, they often contain air. No pore or opening is visible in these hairs even on high magnification, but, if they are dried and then placed on a slide in some such stain as Loeffler's alkaline methylene blue solution, the dye is seen first to penetrate the point of the hair, and afterward gradually to diffuse itself through the remainder of its length. From this phenomenon it appears that there is a minute opening at the point of the nettling hair, although it cannot be visually distinguished. When suspended in a fluid, individual nettling

* Such is the degree of descriptive specialization shown in these peculiar elements, that the term "hair" furnishes but an inadequate conception of their character. On the ground of terminology, however, the term "hair" seems justifiable and the terminology of Fernald and Kirkland in regard to these elements will accordingly be followed.

hairs appear to the unaided vision as scintillating points. In the dry state large masses of them form a fine brown powder, which is very light and easily blown about.

The nettling hairs develop upon the caterpillar. Although Fernald and Kirkland and others state that the nettling hairs are present upon the caterpillar only after the last two molts in the spring, they are, nevertheless, demonstrable much earlier. The two velvety brown spots, which appear on the dorsal aspect of the fifth and sixth segments after the first molt, and while the caterpillars measure but four or five millimeters in length, are found to consist of nettling hairs. Sections of these small caterpillars show the anatomical relations of these hairs, and when the latter are rubbed upon the skin a dermatitis is produced. The caterpillar is thus demonstrated to be poisonous at a very early stage in its development. The young caterpillars hibernate in colonies in the winter webs which are found on the tips of twigs. These webs contain, in addition to the young caterpillars, the skins of their various molts, and may produce irritation if torn open. The two brown spots situated on the back of the caterpillar are in reality two pairs of subdorsal tubercles. They are likewise found on the fifth and sixth segments after each succeeding molt up to the last two spring molts, when they are present on all segments from the fifth to the twelfth, inclusive. Patches of nettling hairs are at this time also found just below the tufts of white branching hairs on the lateral tubercles of the same segments. The increase in the production of nettling hairs at this stage makes the caterpillar "poisonous" to a degree much greater than in any of its preceding molts.

The nettling hairs developing upon the caterpillar may eventually be widely disseminated from their original source. In the process of manufacturing the cocoon, the hairs are all rubbed off the caterpillar and enter into the structure of the cocoon. It is unquestionably by reason of the nettling hairs enmeshed in and adherent to cocoons that a dermatitis so often follows contact with them. Since severe dermatitis has frequently followed the handling of cocoons over a year old,

it is evident that the irritating substance connected with the hairs is, under ordinary conditions, extremely slow to disintegrate.

Nettling hairs are found mingled with the long hairs of the brown tuft on the tail of the moth where they are most numerous near the distal ends and are not found near the roots of the long, coarse hairs. Although the nettling hairs are present in large numbers, especially on female moths, which possess a larger and thicker tuft on the tail, I have been unable to demonstrate that they have any constant anatomical relation to the body of the moth. Fernald and Kirkland, since they found these elements in variable numbers and irregularly distributed over the moths, concluded that they probably become entangled among the scales of the moth as it works its way out through the cocoon, and that thus all the nettling hairs are primarily derived from the caterpillar. The tuft of thick brown hair on the tail of the female is deposited in the form of a felt-work around the eggs as they are laid on the under surface of leaves. As this material contains many nettling hairs, it also is capable of producing the typical dermatitis.

Since the nettling hairs, once they are dislodged from the caterpillar, are blown about by any slight current of air, it seems beyond question that a characteristic eruption may occur without actual contact with caterpillar, moth, or nests. If a susceptible individual stand during a slight breeze beneath a tree well infested with these caterpillars, this fact will be satisfactorily demonstrated. A rash will appear on the exposed parts very soon afterward, and one may experience intense itching almost immediately. The lodgment of the nettling hairs upon the underclothes as they are hung to dry is probably also one of the common sources of the rash. To remedy this the underclothes may be hung right side out instead of being turned as is customary, or they may be sent to a non-infested district to be laundered.

In order to determine the anatomical relation of the nettling hairs, serial sections were made of several of those

segments of the caterpillar which bear the velvety brown patches corresponding to the subdorsal and lateral tubercles. These hairs are found with points inserted in protuberant rounded sockets with which this portion of the cuticle is studded (see Plate, Fig. 3). The sockets are rather closely set but usually not in actual contact one with another. The number of nettling hairs to each socket varies within wide limits. Some sockets hold but three while others hold as many as twenty. Beneath the areas upon which the nettling hairs develop, the eperdermis is modified and is represented by a large mass of cells. These cells are long and fusiform with their long axes perpendicular to the surface of the cuticle. The nucleus in each cell is nearer the extremity away from the surface and the major portion of the cytoplasm is in the form of a long process which extends into the socket which holds the nettling hairs. Whether these groups of cells are of the nature of poison glands or simply the formative cells of this type of hair has not been determined. There is nothing distinctive as regards the microchemical affinities of these cells when compared with cells at the base of the coarse hairs. On the other hand the granular material within the nettling hairs simulates very closely that found within the coarse hairs notwithstanding that one is poisonous and the other not. The homogeneous cuticular layer is continuous over every part of the sockets so that there is no apparent communication between the cells on the interior and the points of the nettling hairs. The significance of the collections of cells beneath the areas bearing the nettling hairs might be understood if stages could be obtained in which these hairs were in the process of development.

There has been considerable conjecture as to whether the dermatitis is due to the mechanical action of the nettling hairs or to a poisonous substance conveyed by them. Fernald and Kirkland submitted material consisting of hairs, cocoons, and molted skins to Mr. F. J. Smith, chemist of the Gypsy Moth Committee. His notes are as follows:

"I made a number of extracts of the hairs with each of the reagents mentioned below, some of the extracts being of the hairs alone, others of the molted skins, and still others of the cocoons which contained hairs in great numbers. The reagents used were: water, alcohol, ether, chloroform, petroleum ether, acetone, acetic ether, dilute sulphuric acid, dilute caustic potash. I tested each of the extracts after digesting for some hours, and in each case they nettled the skin. On the other hand, the *filtered* extracts (freed from hairs) caused no irritation of any sort when applied even where the skin was broken. Careful chemical tests failed to show the presence of any organic acids or alkaloids. Hence I am led to believe that the irritation is of a mechanical nature, caused by the brittle, finely barbed hairs, and not due to a toxic principle."

This analysis would indicate that the action of the nettling hair is a purely mechanical one. These results, however, appeared far from conclusive when the degree of the reaction in the tissue around these extremely minute elements was considered.

In order to follow up this question, controls of the nature of purely mechanical agents were sought. Fine glass wool was comminuted between two glass slides and then rubbed upon a small area of the skin of the upper arm. This was followed by the appearance of numerous minute red spots. There was a slight soreness over this area, suggestive of the presence of minute foreign bodies in the skin. The red spots persisted for twenty-four hours or more.

The tufts on the back of a tussock moth caterpillar were examined and found to consist of long, sharp-pointed hairs with sharp barbs projecting at intervals along their entire length. These hairs were of the same general type as the nettling hairs of the brown-tail moth, but were many times as long. When such hairs from the tussock moth caterpillar were rubbed upon the skin, several minute reddish points appeared. There was a barely perceptible prickling sensation but no itching. Another person inoculated in a similar manner showed no lesion whatever. Thus neither finely comminuted glass wool nor the sharp-pointed, barbed hairs of a tussock moth caterpillar when rubbed into the skin produced any process resembling in character the brown-tail moth dermatitis.

At this time the accidental discovery of a peculiar reaction, which takes place when the nettling hairs of the brown-tail moth are mingled with blood, indicated the presence of a soluble chemical substance in connection with them. If a number of nettling hairs are placed in a drop of blood between a slide and cover-glass, an immediate change takes place in the red blood corpuscles. They at once become coarsely crenated and the rouleaux are broken up in the vicinity of the hair. The corpuscles decrease in size and the coarse crenations are transformed into slender spines which rapidly disappear leaving the corpuscles in the form of spheres, the light refraction of which contrasts them sharply from the normal corpuscles. The change of form, in addition to a slight shrinkage, causes the red blood corpuscles to appear much smaller than normal. This reaction takes place so rapidly when the fresh, active, nettling hairs are used that the eye cannot follow its various stages. However, by treating these hairs in various ways, the time of this reaction may be slowed so that all stages of transformation may be seen. The reaction always begins at the basal sharp point of the hair. It was thought that this might possibly be a purely physical phenomenon, but this is disproved in various ways. A variety of minute foreign bodies were mingled with the blood in a similar manner. Such material as minute particles of glass wool, the barbed hairs of the caterpillar of a tussock moth, and the other coarser hairs of the brown-tail, all failed to produce any effect on the red blood corpuscles.

The next step was to determine whether the nettling hairs could be inactivated by heating. After baking for one hour at 100° C., they gave a typical reaction with red blood corpuscles and produced a dermatitis when rubbed upon the skin. Baked for one hour at 110° C., they still gave the typical reaction with red blood corpuscles and produced a typical dermatitis when rubbed on the skin. However, after being baked an hour at 115° C., the nettling hairs no longer affected the red blood corpuscles and when even rubbed into

the skin now failed to produce a typical dermatitis, but only a slight redness which was scarcely discernible and which was probably comparable with that produced by the penetration of the epidermis by minute foreign bodies such as particles of glass or the barbed hairs of other caterpillars. The degree of heating does not, in this instance, affect the structural integrity of the nettling hairs, for they appear unchanged even after baking an hour at 150° C.

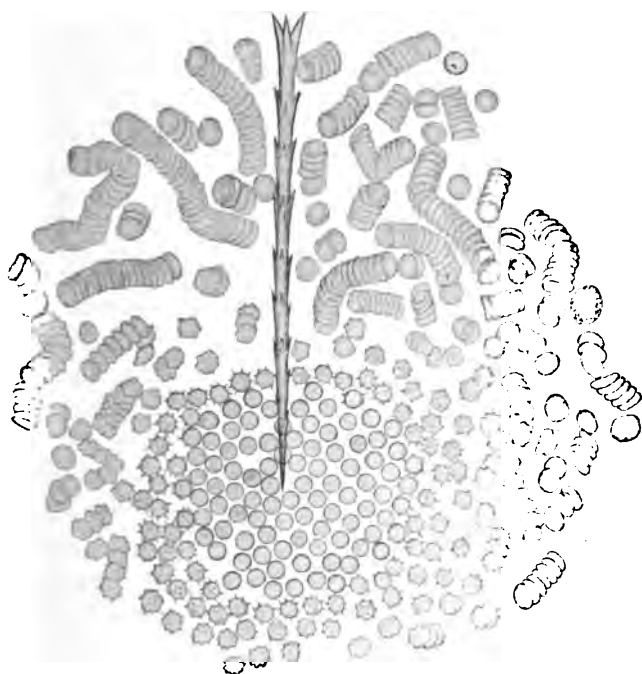
The experiment of heating the nettling hairs at various temperatures proves conclusively that their action upon the skin is not purely a mechanical one, but that it depends upon the presence of a chemical substance which is destroyed at high temperatures. The failure of the nettling hairs, after being heated to 115° C., to give the characteristic reaction with red blood corpuscles, together with the fact that they no longer produce the typical dermatitis, suggested the possibility that this peculiar reaction might serve as an index to their toxicity. This was put to the test in subsequent experiments, and this inference has been borne out.

In order to determine the solubility of the irritating substance conveyed by the nettling hairs, the effect of various solvents was tried both at room temperature and heated. For the time being the presence of the poison in the solvent was disregarded and the reaction of the nettling hairs with the red blood corpuscles and upon the skin was determined after they had been placed under the influence of the solvent. By so doing it was believed that it would be possible to estimate at least the relative degrees of solubility of the irritating substance.

Treated with alcohol, acetone, chloroform, and ether, the nettling hairs remained active both as regards the test with red blood corpuscles and the inoculation of the skin, whether boiled for a short time or kept for days at room temperature.

The nettling hairs remained active after being boiled in pyridin, which boils at a temperature between 106° and 108° C.

Kept in pure glycerine or in equal parts of glycerine and distilled water for several days, the nettling hairs remained



A sketch showing the effect of the netting hairs of the brown-tail moth upon mammalian red blood corpuscles.

active. They were also active after heating at 110° C. in pure glycerine, but after heating at 115° C., they failed to react. As this was the approximate temperature at which they were inactivated by dry heat, it seems certain that the poison is destroyed at this temperature.

The nettling hairs also remained active when kept for several days in glacial acetic acid, in one-half per cent acetic, and in both one per cent and one-tenth per cent aqueous solutions of hydrochloric acid. The reaction with the red blood corpuscles was delayed for a short time after the soaking in acids, but afterward went on undiminished. This retarding of the reaction is probably due to the fact that several moments must elapse before the acidity of the hairs is neutralized by the blood in which they are placed. The retardation is more pronounced after strong than after weak acid solutions and, if the hairs are subsequently thoroughly washed in water, there is no slowing of their reaction with the red blood corpuscles.

In distilled water the nettling hairs remained active after a period of eighteen days even though during this time they were centrifugalized and washed several times, in addition to being placed in the incubator at 38° C. for sixteen hours. When warmed in distilled water to 50° C. they were still active, but when warmed to 60° C. they immediately failed to react. Since the substance which gives the reaction withstands much higher temperatures, it seems reasonably certain that it is dissolved out in water raised to this temperature.

Inasmuch as the poisonous substance was evidently soluble in fluid blood at room temperature, it seemed plausible that it might be soluble in dilute alkaline solutions. This was found to be the case. Both one per cent and one-tenth per cent solutions of potassium hydrate and sodium hydrate in distilled water were used. The nettling hairs, after remaining over night in these solutions at room temperature, failed to act either on the skin or on the red blood corpuscles.

From these data it seems necessary to conclude that the nettling hairs possess a substance which acts as an irritant to

tissues (epidermis), which is relatively stable, being destroyed by heating at the temperature of 115° C. or over, and which is quite soluble in dilute alkalies at room temperature or in water warmed to 60° C. I have been unable to demonstrate whether this substance is located within the nettling hair or carried upon the surface at its point. If it is true that the wall of the nettling hair is perforated at its point, as indicated by the penetration of staining fluids, it seems not unlikely that the poisonous substance is contained within the chitinous walls. This is borne out by the activity of these hairs after soaking two and a half weeks at room temperature in water — a reagent which is known to be a solvent when raised to 60° C. — for it seems improbable that so small an amount of the irritating substance, as there must necessarily be, could remain undissolved for this length of time if smeared on the surface of the hair. Furthermore the specific reaction with the red blood corpuscles always begins at the point of the hairs except in instances in which they are broken, when the reaction takes place rapidly about the point of fracture. If the irritating substance was secreted, as certain observers have believed, by the two retractile tubercles on the hinder segments and smeared by the movements of the caterpillar over its hairs, the coarse hairs also would have nettling properties and react with the red blood corpuscles; but such is not the case. It is probable that protection afforded the irritating substance by its inclusion within chitinous walled tubes and also its chemical stability account for the fact that the nettling hairs remain active for long periods of time unaffected by natural influences such as fluctuations of temperature or repeated wetting and drying.

Since it was impossible to obtain caterpillars in sufficiently large numbers for an exhaustive study into the chemical nature of the irritating substance, a preliminary analysis of the material at hand was kindly undertaken by Dr. Carl L. Alsburg of the Department of Biochemistry, Harvard Medical School. His notes are as follows:

“ An extract of all the hairs furnished me was made in distilled water at 60° C. It was faintly clouded and slightly tinged brown. It gave no

Millon or Biuret reaction, and could not, therefore, have contained any appreciable quantity of proteid. It showed no coagulation on heating. Its reaction was slightly acid. With saturated aqueous picric acid it gave a fine not very abundant granular precipitate. With a twenty-five per cent solution of phosphotungstic acid in five per cent H_2SO_4 there was an abundant heavy precipitate which formed gradually. With tannic acid there was an abundant brown flocculent precipitate. Silver nitrate caused a slight brown precipitate. Potassium mercuric iodide, barium chloride, and mercuric chloride merely produced turbidity. The solution did not reduce Fehling's solution either before or after boiling with dilute mineral acid. As the quantity of material used in the last two tests had to be very small, the results must be taken as provisional. The small quantity of material available moreover made it impossible to test the toxic action of the precipitates formed by the various reagents. There is, therefore, no guarantee that the substance or substances precipitated by phosphotungstic acid, etc., actually are the active principle."

The difficulties in the isolation of a chemical substance of this nature are great, and this undertaking has been deferred until another season when it may be possible to obtain material in sufficient amount with which to work advantageously. In making extracts from the nettling hairs other substances besides the irritating principle will probably be found, which will have to be eliminated. In this instance the biological test will undoubtedly be of value.

The possibility of the occurrence within the body of the caterpillar of an irritating substance identical with that found in the nettling hairs has been considered. If a brown-tail caterpillar be laid open and precautions are taken not to introduce any nettling hairs into its tissue, its fluids will be found to be highly poisonous if placed on a slight excoriation of the skin. The part commences to itch at once and the skin becomes elevated, white, and edematous, and a large wheal develops. The lesion has a well defined, abrupt edge and spreads for a radius of one centimeter or more about the scratch. A reddish petechial flush appears over a wide area of skin about the wheal. At one-half or three-quarters of an hour after the inoculation, the process is at its height, and after an hour the border of the lesion is not so sharply defined. The edema subsides gradually, giving place

to diffuse redness and the skin over this area feels slightly sore for several hours. In this manner the fluids of the caterpillar may be demonstrated to be poisonous, but the irritation is due to a substance quite different from that found in the nettling hair. This is proved by the failure of the fluid from the caterpillar to produce, when mingled with mammalian blood, any characteristic change in the red blood corpuscles. The reaction of the human skin in this instance is much more severe, but, on the other hand, is of much shorter duration than is the case of the lesions produced by the nettling hairs. It is possible that the fluids of other caterpillars, usually regarded as innocuous, would be found poisonous if rubbed into excoriations of the skin, but this was not tried.

The pathological processes produced by the nettling hairs of the brown-tail moth were studied in both human and animal tissues.

The reaction of the human skin to the nettling hairs of the brown-tail moth varies greatly with different individuals. When these hairs are rubbed upon the skin there is immediately, with most persons, considerable itching and the inoculated area rises up in the course of fifteen or twenty minutes in the form of a wheal about which there is considerable reddening that changes to white when the skin is stretched. The reaction, however, is not always so prompt to appear. The severest process observed showed nothing for a period of eight hours after the inoculation. The nettling hairs even when rubbed into the skin of other individuals produce only slight reddening or perhaps very slight elevation and practically no discomfort. Whether or not decreased alkalinity of the blood forms a factor in the insusceptibility of these persons is only a matter of conjecture. The blood of all persons and of all laboratory animals thus far tried has reacted in a typical manner to the nettling hairs.

The dermatitis, as it occurs naturally, is of two types according to the manner of acquisition. If, for instance, a caterpillar is felt crawling over one's neck and is thereupon

slapped or crushed and the part afterward thoroughly rubbed and scratched, a marked local dermatitis develops in which the lesions are confluent. There is local reddening and thickening of the skin with the formation of papules or vesicles, as the case may be. A patient in this condition is liable to seek the advice of a physician. On the other hand, if the nettling hairs are distributed by a breeze to underclothes as they hang drying, the dermatitis which results from wearing these clothes is of the nature of a scattered urticarial rash. The lesions in such a case are in the form of small discrete papules which, if not scratched, often show at their summit a tiny vesicle filled with clear fluid. They are generally more numerous on parts of the body where the skin is soft. Many persons having such rashes never consult the physician. Warm, muggy weather aggravates the condition; the reason is that the epidermis is then kept moist by perspiration and becoming softer favors the penetration of the nettling hairs. In dry, cool weather the epidermis is less easily penetrated.

The duration of this form of dermatitis may be long on account of repeated inoculations. The individual lesions usually, however, heal in from a week to ten days. The severe localized form of dermatitis is perhaps more prevalent in May and June, as it is then that the caterpillars are reaching their maturity. The form of dermatitis in which the rash is scattered is common when the moths are flying in large numbers (July), as well as earlier in the season of the caterpillars. A dermatitis may be acquired at any season of the year by the handling of large numbers of nests or cocoons. The wearing of an undergarment which had been packed away a year since its contamination with nettling hairs has often been sufficient to produce dermatitis.

The material on which the histological study of the dermatitis is based consists in part of a piece of skin excised from the upper arm of the author twenty hours after inoculation with the nettling hairs of a caterpillar collected early in March. The tissue after fixation in Zenker's fluid was imbedded in paraffin and serial sections made. Without

serial sections it would be difficult, except perhaps by accident, to find the nettling hairs in the tissue. Material for supplementary study was furnished by lesions produced experimentally in animals.

The lesions in the human skin may be summed up as follows:

The nettling hairs are found imbedded at various depths; some penetrating in a direction oblique to the surface are situated superficially in the epidermis; others have penetrated the entire thickness of the epidermis; and others have passed through the epidermis and have penetrated the corium for nearly their entire length. There is necrosis of the epidermis around the nettling hair, and in most instances there is exudation of fluid into the epidermis so that a tiny vesicle is formed. The contents of the vesicle consist of clear fluid in which are disintegrating epithelial cells, a few large mononuclear cells (phagocytes), and numerous eosinophiles which in many instances are completely disintegrated. The latter cells are found constantly and appear to be the earliest cells to invade the epidermis about the foreign hair. Many occur with irregular pseudopodia-like processes as though fixed when in ameboid motion. The disintegration of these cells forms masses of eosin-staining granules which are found in the spaces in the epidermis. In certain instances the space in the epidermis occupied by the exudate appears continuous with a dilated lymphatic. There is marked inflammatory reaction in the corium as shown by the presence of large collections of cells about the blood vessels. These collections of cells consist of large mononuclear (or transitional) cells which are often phagocytic, of lymphoid cells, and of numerous eosinophiles. An occasional polynuclear leucocyte is found.

Thus the process in the human skin consists of the penetration of the nettling hairs into or through the epidermis, the necrosis of contiguous epidermal cells, and exudation into the epidermis, a constant element in which are numbers of eosinophile cells. There is undoubtedly local congestion and edema of the corium, but this did not appear to any marked degree in the sections studied.

As the amount of inoculable material at hand was small, but few animal inoculations were performed. Mice were inoculated subcutaneously and intraperitoneally with both active nettling hairs and nettling hairs inactivated by soaking in a dilute solution of an alkali. The nettling hairs were made practically sterile by boiling for ten minutes in strong alcohol, a process which does not destroy their specific action, and then suspended in a .6 per cent solution of sodium chloride. Probably on account of the tendency of the fresh, active nettling hairs to rise to the surface of the suspension fluid, only a small number were injected, for they were found with difficulty in the tissue. The inactivated nettling hairs, on the other hand, were evenly distributed in the suspension and were found in the tissue in large numbers. Some of the mice, more especially those receiving the active nettling hairs, showed evidence of intense itching of the skin at the site of inoculation. Fifteen hours after the inoculation, a slight subcutaneous swelling, more marked in certain of the mice which had been inoculated with active nettling hairs, was distinguishable.

A mouse killed twenty-four hours after inoculation with active nettling hairs showed an ill-defined, pinkish tinged area of subcutaneous edema at the site of injection. Stained sections of this area show skin as well as subcutaneous tissue distended with fluid exudate. Large numbers of polynuclear leucocytes are present, scattered through the tissue. No nettling hairs are present in that portion of the lesion from which the sections are taken. The peritoneum presented no gross lesion. Stained preparations of the mesentery show numerous minute inflammatory foci, consisting of aggregations of phagocytic cells, intermingled with lymphoid cells. There is also a small number of polynuclear leucocytes scattered through the mesentery. The nettling hairs are often apparent within these foci of inflammation.

A mouse killed twenty-four hours after inoculation with the inactivated nettling hairs presented a small well defined, dull reddish area on the inner aspect of the skin at the site of inoculation. Sections of this lesion show enormous

numbers of nettling hairs imbedded at about the level of the superficial muscle layer, the fibers of which are here either degenerating or necrotic. Some fibers are actually pierced by the hairs. In the immediate vicinity of the injury are large numbers of polynuclear leucocytes, many of which are disintegrated. There is practically no distension of the surrounding tissue with fluid. On the peritoneum of this mouse were several minute whitish flecks apparent to the naked eye. The histological study of the mesentery and omentum reveals very numerous foci of inflammation of the same general character as those found in the mouse inoculated with the active nettling hairs. These inflammatory areas consist chiefly of collections of phagocytic cells grouped about the nettling hairs. Lymphoid cells are not so numerous as in the lesions of the mesentery in the preceding case.

In mice killed forty-eight hours after the inoculation the subcutaneous lesions were scarcely distinguishable and, on account of the difficulty of orientation, the sections made did not pass through the lesions. The mesenteries, in addition to the cells already described, show numerous stellate cells, probably fibroblasts.

The results of these few animal inoculations show clearly one point of difference between the lesions produced by the active nettling hairs and the lesions produced by those which have been inactivated. The lesion produced by the subcutaneous inoculation of the active nettling hairs shows a much greater amount of fluid exudate than is the case in the lesion produced by inactivated nettling hairs. This fact tends to support the view that the action of the nettling hairs is not purely a mechanical one.

SUMMARY AND CONCLUSIONS.

The most important facts thus far ascertained concerning the brown-tail moth dermatitis may be summarized as follows:

The lesions are produced by the penetration into the epidermis of peculiarly modified microscopic hairs, the nettling hairs, which are sharply pointed and barbed for their entire length. The other hairs of both the caterpillar and

the moth do not produce any dermatitis. These nettling hairs develop on the caterpillar but are also found, as the result of direct transference, on the cocoons, moths, egg masses, and in the winter webs, and are blown about in the air. They develop on the subdorsal tubercles of the fifth and sixth segments of the young caterpillars, but are much more numerous as the caterpillars attain their growth, being then found on the subdorsal and lateral tubercles from the fifth to the twelfth segment, inclusive. The caterpillars are then very poisonous.

The nettling hairs, which from their shape are perfectly adapted for penetration, possess in addition an irritating substance which is undoubtedly an important factor in the production of the dermatitis.

This substance may be destroyed by heating the nettling hairs at 115° C., either in a fluid or with dry heat, or it may be extracted from them by certain solvents such as dilute solutions of alkalis at room temperature, or water heated to 60° C. Nettling hairs inactivated by either of these measures produce no more than a slight redness when rubbed into the skin, and probably act then merely as foreign bodies.

An index for the presence of the irritating substance is found in a peculiar reaction which takes place when the active nettling hairs are mingled with a drop of blood between slide and cover-glass. This reaction begins about the point of the hair but spreads rapidly so that a large area is involved. The first change is the breaking up of the rouleaux of red blood corpuscles. The corpuscles then become coarsely crenated, later are spherical with slender spines protruding from the surface, and finally appear perfectly spherical and closely packed. If the irritating substance has been previously either inactivated by heat or extracted from the nettling hairs they no longer give this reaction with the red blood corpuscles.

The dermatitis produced by the nettling hairs is of two types, dependent upon the number of these elements penetrating a given area. The severe type is usually due to actual contact with caterpillars; the milder scattered rash is

due to nettling hairs blown in the air and lodging on the skin or on the undergarments as they hang drying. The pathological process in the skin consists of necrosis of the epidermal cells around the nettling hairs, the formation of spaces or microscopic vesicles in the epidermis at the site of injury, and inflammatory changes about the vessels of the corium.

Mice inoculated with active nettling hairs present lesions characterized by a large amount of fluid exudate, while those inoculated with inactivated nettling hairs show inflammation of the nature of a reaction due to the mechanical injury brought about by these elements.

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DESCRIPTION OF PLATE.

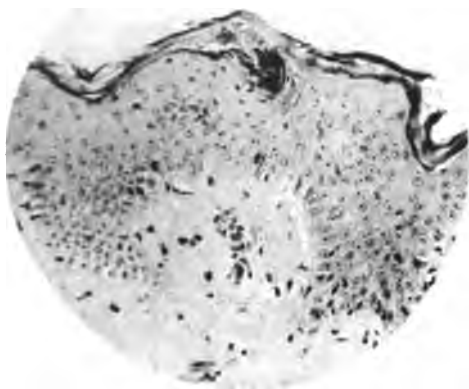
FIG. 1. — Human skin excised twenty hours after being rubbed lightly with a small brown-tail caterpillar. The nettling hair has penetrated only the superficial layers of the epidermis. About it a minute quantity of fluid separates the horny layer; its point is embedded in a mass of deeply staining coagulum in which are necrotic epidermal cells.

FIG. 2. — A section from another portion of the same piece of excised skin. The point of the nettling hair has, in this instance, passed through the epidermis and penetrated the corium. A more or less conical mass of sequestrum marks its course. A small number of leucocytes are present in the tissue about the injury. (Since the point of the nettling hair showed but faintly in this photograph its outline has been sharpened for purposes of reproduction.)

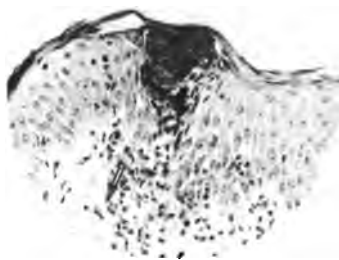
FIG. 3. — A section of a caterpillar showing the nettling hairs as they are developed upon its skin. An area of cuticle is shown upon which are numerous rounded sockets each of which bears a number of nettling hairs with their points inserted in the sockets. Below this area is a mass of long fusiform cells, and to the left is a large deeply stained cell marking the insertion of one of the coarse hairs of the caterpillar.

FIG. 4. — A minute cavity formed about a nettling hair which has penetrated the epidermis. Adherent to the nettling hair, the point of which is directed to the right, is a mass of deeply stained coagulum. The fluid in the cavity contains degenerated cells. (Magnification greater than in other figures.)

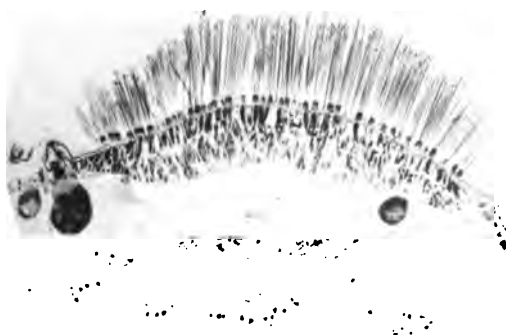
FIG. 5. — A lesion showing the effect of excoriation of the affected area. A minute portion of the epidermis is necrotic and is included in a small crust. The nettling hair does not appear and has probably been rubbed away. There is considerable cellular exudation into the subjacent corium.



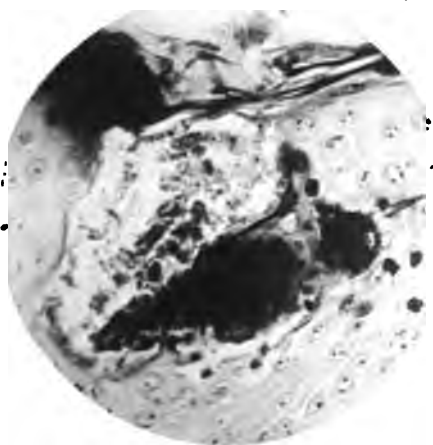
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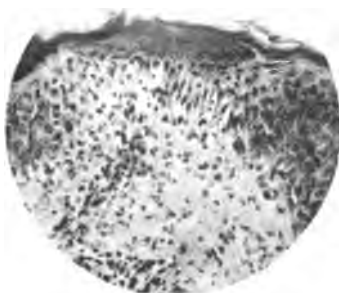
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5

Tyzer.

Brown tail moth.

TRYPANOSOMES OF THE TRUMPTER HORNBILL (BYCANISTES
BUCCINATOR).*

BY

THE LATE

J. EVERETT DUTTON, M.B., *Vict.*, JOHN L. TODD, B.A., M.D., *McGill*,

AND

E. N. TOBEY, A.M., M.D., *Harvard*.

(*From the Runcorn Research Laboratories of the 'Liverpool School of Tropical Medicine.'*)

Two distinct trypanosomes, one small, the other large, were seen in a bird of this species shot near Coquilhatville in the Congo Free State in 1904.†

The smaller trypanosomes (Figs. 1, 2, 3, 4, 5, 6) were by far the most numerous. Their appearance in stained specimens varies very considerably so that it is possible to describe three distinct types, although forms intermediate between them can be distinguished. These may be called the "slender" (Figs. 1, 2), "broad" (Figs. 3, 4), and "stumpy" (Figs. 5, 6) forms in accordance with their general appearance. To some extent the structure of these forms varied with their dimensions. The stouter forms usually stained more lightly, had the looser cytoplasm, had no vacuole at the posterior extremity, and their large nuclei did not extend completely across their bodies.

This association of qualities was not constant, however, and all gradations were seen between forms possessing them and the, as a rule, darkly-staining and more compact slender forms. All these forms are therefore considered to be merely variations of one parasite which may be described as follows:

The blepharoplast stains very densely and more darkly than either nucleus or flagellum. Its position may vary from the extreme posterior extremity, particularly in the

* Received for publication Dec. 18, 1906.

† The material on which these observations are based was collected by the Expedition of the Liverpool School of Tropical Medicine to the Congo. Fresh specimens were cursorily examined at the time the parasite was found, while dried films were made, stained with a modification of Romanowsky's method and preserved for a future examination. The appearances described in the text and illustrated in the plate were seen in these specimens with a Zeiss, two millimeters, apochromatic objective, fitted to a tube length of two hundred and fifty millimeters, and a number eight eye piece. The figures are based on careful measurements and were made without drawing apparatus.

"broad" and "stumpy forms," to a spot 1μ or more from the end of the parasite (Fig. 1). It is oblong in shape and is seen to be granular, and in several specimens at least four granules, sometimes arranged in pairs, could be counted (Fig. 6). Although the arrangement of these granules occasionally suggested commencing division, none of the ordinary longitudinal division forms were seen. The blepharoplast may be placed longitudinally, obliquely or transversely in the parasite. Just anterior to it there is often a well-defined vacuole or, when that is absent, a more lightly stained area.

The nucleus frequently extends completely across the body of the parasite, and is almost always surrounded by an area stained more lightly than is the remainder of the parasite. The relation between the size of the nucleus and the type of parasite varies considerably; as a rule in the "broad" and "stumpy" forms the nucleus seems relatively larger, of looser texture and stains more lightly. Chromatic granules, number undetermined, occur in the nucleus of each type of trypanosome (Fig. 1).

In several preparations (Figs. 3, 6) two small darkly-stained and closely-opposed chromatinic granules surrounded by a pinkish area occur in close connection with the nucleus, but just outside the nuclear membrane. In one instance a well-marked chain of oblong twin chromatinic granules runs forward, in an unstained area, from the anterior edge of the vacuole for about a quarter of the distance between the blepharoplast and the nucleus. This structure recalls a similar appearance observed in *Trypanosoma karyozenktion*.¹

Whether staining deeply or lightly the cytoplasm of the small trypanosomes is always granular, but the fineness of the granules varies greatly. Well-marked striations of the protoplasm occur in each form, although they are best seen in the stouter ones (Figs. 3, 4, 5).

The striations are evidently a superficial structure, and are usually wound spirally about the parasite. In some specimens a single striation can be followed for almost two complete turns (Figs. 4, 5). From their arrangement it is

very difficult to estimate the number of striations, but there seem to be about eight (Figs. 4, 5).

The whole parasite is enclosed in a pink-staining periplast which can be plainly seen at the posterior extremity, and in some parasites all along the edge of the body where it is unobscured by the undulating membrane (Fig. 3). As is shown in Fig. 4, the striations are continued in the periplast at the posterior end of the parasite after the darkly-staining cytoplasm has ended. The ample undulating membrane seems relatively widest in the "slender forms."

The usual dimensions of each form are given in the following table :

	Stumpy Form.	Broad Form.	Slender Form.
I*	1.0 μ	1.6 μ	1.6 μ
II.	7.0 μ	8.0 μ	10.4 μ
III.	2.0 μ	2.4 μ	2.0 μ
IV.	7.3 μ	10.4 μ	7.2 μ
V.	7.8 μ	8.0 μ	9.6 μ
VI. (breadth)	7.0 μ	4.8 μ	2.8 μ
VII. (total length)	25.1-30 μ	30.14 μ	30.8 μ

* I. Posterior extremity to center of blepharoplast.

II. Center of blepharoplast to posterior border of the nucleus.

III. Length of nucleus.

IV. Anterior border of nucleus to anterior extremity of the body.

V. Length of the free flagellum.

VI. Width of the body, without undulating membrane, at its widest part.

VII. Usual total length of the parasite.

Only two examples of the large trypanosome (total length about 64 μ) were obtained. Unfortunately both are so obscured by surrounding red cells that it is impossible to reproduce them. Their blepharoplast is placed much nearer to the nucleus than to the posterior extremity (and in one instance in a vacuole). The nucleus almost extends across the body. The undulating membrane is ample, and the flagellum seems to be comparatively short. The body of

the parasite is striated longitudinally. At the level of the nucleus only seven of the probably eight striations could be counted. In one parasite appearances resembling longitudinal striations were present in the undulating membrane. The dimensions of this type are, posterior extremity to center of blepharoplast 28.3μ (in one parasite only 17μ), center of blepharoplast to posterior border of nucleus 3.3μ , length of nucleus 3.3μ , anterior border of nucleus to termination of body 18.3μ , free flagellum 8.3μ , width 5.8μ .

At Coquilhatville lack of time prevented a careful examination of the parasite in fresh preparations. Some months later another hornbill was shot and its blood was found to contain trypanosomes resembling the small type described above. Fresh coverslip preparations of blood were kept at room temperature (22°C.) and watched for some hours by Dr. Inge Heiberg. Two hours after the preparation was made, normal parasites were still seen. Occasionally pairs occurred joined by their flagella. After three hours both normal and very short stumpy parasites were seen, while after four hours, longitudinally dividing, spherical and irregular, certainly degenerating, parasites were present.

Eighteen hours after the preparation was made dividing forms, similar to those seen at the fourth hour, were still present, while a stouter trypanosome than those seen at the commencement of the observation now appeared for the first time.

One parasite, watched for the first three hours following the making of the preparation, was seen to undergo the changes illustrated in the accompanying diagram.

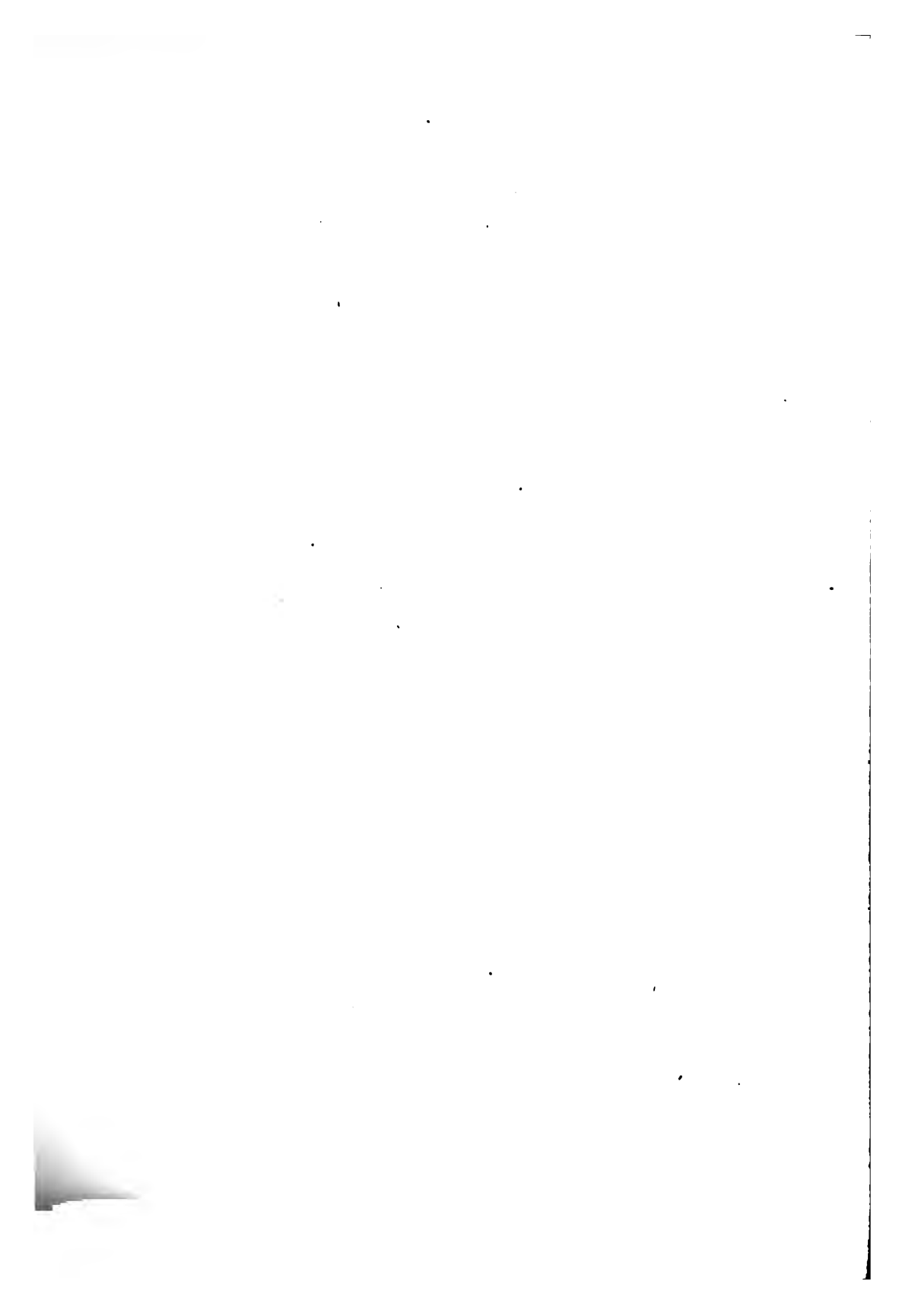


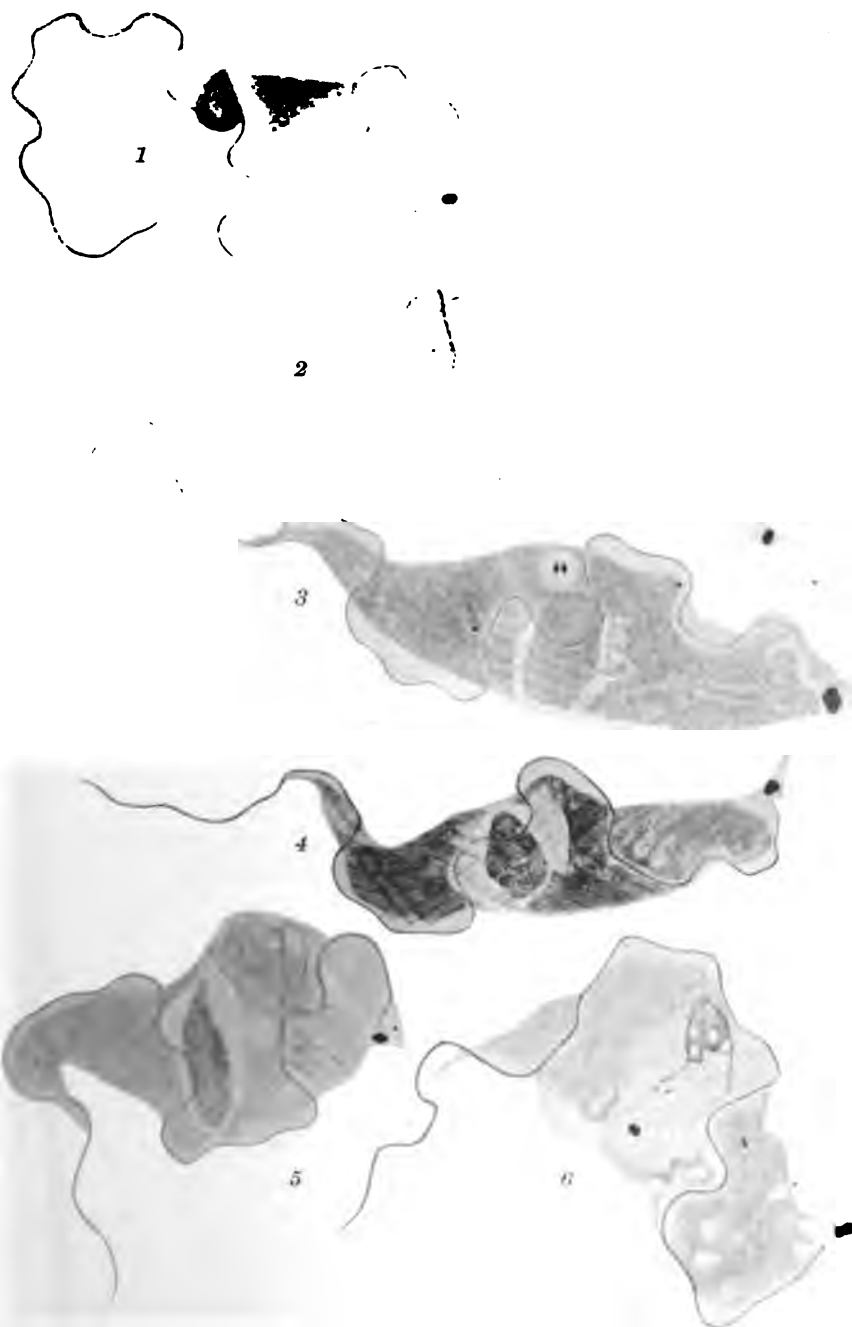
LITERATURE.

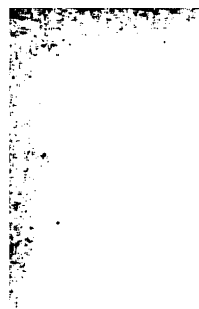
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DESCRIPTION OF PLATE.

- FIG. 1. — "Slender" form.
FIG. 2. — "Slender" form.
FIG. 3. — "Broad" form.
FIG. 4. — "Broad" form.
FIG. 5. — "Stumpy" form.
FIG. 6. — "Stumpy" form; evidently degenerated but produced to show structure of blepharoplast and nuclear granules.







THE URICOLYTIC ENZYME.*

(*Second Contribution.*)

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In my first contribution¹ on this subject objections were raised to the results of others because alkalis were used in the solution of the uric acid added, by which the digestive mixture was rendered alkaline, an alkalinity which by itself at 28° C. in pure watery solution was found sufficient to destroy a large amount of the acid added. This as can be readily seen masks the efficiency of the enzyme since a part of its apparent efficacy may be due to the splitting action of the alkali. Again in the Salkowski-Ludwig method of determining uric acid, sodium sulphide is used with heat for separating the silver from the silver-magnesium urate which also has a tendency to split the uric acid present.

Burian² concedes that in alkaline uric acid solutions the acid is destroyed when oxygen is led through the mixture, but my results have shown that it is also destroyed when no oxygen is employed. In my work the time of digestion was extended to seventy-two hours instead of Burian's ten hours (limit) and the destruction by this means at 38° C. increased with the time of exposure. Schittenhelm³ makes no mention of the effect of the alkali used in solution upon uric acid, hence we assume that he regards it of no importance; yet he uses solutions of sodium hydrate of only .08 per cent and in the same article (on page 164) where cooked enzyme solution is used, which of course excludes all possibility of the digestive action of the ferment, lost one-sixth of the added uric acid, a result which apart from the error in determination shows the destructive effect of the alkali, joined with warmth upon the acid. Hence in my second series of experiments with one exception, to verify the work of Schittenhelm, only those weaker alkalis like disodium phosphate,

* Received for publication Jan. 22, 1907.

sodium bicarbonate, and lithium carbonate were used to dissolve the uric acid when added to enzymatic extracts. As my previous experience showed that a current of air did not materially increase the amount of uric acid destroyed, it was omitted in these experiments. Burian³ employed compressed oxygen in all but two experiments, but as in these the acid was added in substance and not previously dissolved in alkali, the comparison was not a fair one. Under these conditions, however, he found an increased destruction of the acid when oxygen was used over that when it was not. On account of the dangers of cooking uric acid with alkali as mentioned above, the magnesium-silver salt of the acid was always decomposed by hydrogen sulphide unless otherwise mentioned, after being lightly acidified with hydrochloric acid. There is a danger in this method, of course, that some of the uric acid may be thrown down and lost in the silver sulphide resulting, but if filtered hot, the sulphide cooked out with weak sodium carbonate solution, and well washed with hot water, this error is reduced to a minimum. In order to insure the accuracy of the amount of uric acid obtained from the digestive mixtures as well as its purity, it was weighed in many cases after thorough washing with water, alcohol, carbon disulphide and ether, and then its nitrogen determined by Kjeldahl from which by use of the factor three the amount was also calculated. Purification of the final product by solution in strong sulphuric acid (.1 gram unpurified uric acid to two cubic centimeters acid) and reprecipitation by the addition of four times as much water was also attempted, but a distinct loss was always shown: for instance, three-tenths of a gram of Schuchard's preparation treated in this way showed a loss of .0052 gram or 1.7 per cent; .3307 gram of the same preparation treated in the same way showed a loss of .0062 gram or 1.8 per cent—hence this method of purification is not safe without a correction.

Both the methods of Rosell⁴ and precipitation with ammonium sulphate followed by dialysis were used in isolating the enzyme from organs; each has its disadvantages. The former requires long periods of shaking to

remove the ferment, while the latter demands long continuance of the dialysis against running water to remove the salt. With the simple extraction of the organs with chloroform water as used by Burian, I have had no experience. The method of Rosell affords a solution much more free from albuminous material, but apparently purin bases which are liable to be converted into uric acid are well represented in the extract. On account of this extreme sensitiveness of uric acid to alkali and heat, it is very essential that the digestions with their alkaline reaction should be carefully neutralized before an attempt is made to heat them for the removal of whatever albumin may be present.

The effect of alkalis in splitting uric acid. — In order to determine how important a feature the alkali may be in splitting uric acid at 38° C. a series of digestions were prepared with disodium phosphate, sodium bicarbonate, lithium carbonate and weak sodium hydrate of the strength used by Schittenhelm. The uric acid (both Schuchard's and Merck's were used) was rubbed up with successive portions of the solvent (alkali and four hundred cubic centimeters water) and removed to a Mason jar which was tightly stoppered and kept on ice or at 38° C. as the case might be.

The results of these digestions may be seen in Table I. which follows:

TABLE I.

No. of Experiment.	Amount and Nature of Alkali.	Amount of Water.	Time of Digestion.	Amount of Uric Acid Recovered (by Weight).	Amount of Uric Acid Recovered (from N).	Per cent of Uric Acid Destroyed.	Remarks.
9 A..... B.....	11.35 g. Na_2CO_3 . " "	400 cc. " "	72 hrs. at room temp. 72 hrs. at 38°.2502 g. .1326 g.	16.6 49.9	Ag. removed with H_2S .
10 A..... B.....	15 g. Na_2CO_3 . " "	400 cc. " "	72 hrs. at room temp. 72 hrs. at 38°.2575 g. .1747 g.	14.1 41.7	
11 A..... B..... C.....	5 g. Na_2HPO_4 . " " 5 g. Rochelle.	800 cc. " " " "	66 hrs. on ice. 66 hrs. at 38°. " " "	.1917 g.1392 g.1814 g. .1344 g.	36.1 39.5 55.2	
12 A..... B.....	6 cc. N. NaOH . " "	400 cc. " "	43 hrs. on ice. 43 hrs. at 38°.	.1724 g. .0684 g.	.1722 g. .0667 g.	42.6 77.7	
13 A..... B.....	6 cc. N. NaOH . " "	400 cc. " "	43 hrs. on ice. 43 hrs. at 38°.	.1432 g. .0885 g.	.1308 g. .0882 g.	53.4 70.6	
14 A..... B.....	1 g. Li_2CO_3 . " "	400 cc. " "	41 hrs. on ice. 41 hrs. at 38°.	.132 g. .0989 g.	.120 g.	57 67	
15 A..... B.....	1 g. Li_2CO_3 . " "	400 cc. " "	17 hrs. on ice. 17 hrs. at 38°.	.2022 g. .1617 g.	30.2 46.1	
16 A..... B.....	1 g. Li_2CO_3 . " "	400 cc. " "	5 hrs. on ice. 5 hrs. at 38°.	.0780 g. .025 g.	73.7 91.6	Ag. removed with Na_2S .

The amount of uric acid added in every case was .3 grain.

From a perusal of the above figures it may be seen how sensitive uric acid is to the action of alkalis; even in the cold the action does not cease, although of course it is not so effective as with warmth. In order to guard against the possibility that the isolated acid might not be fully destroyed by the Kjeldahl process as suggested by Kutscher and Steudel,⁵ .3 gram of the same acid employed in the experiments was converted to ammonium sulphate and its nitrogen determined, which gave .1001 gram or 33.36 per cent of nitrogen, the equivalent of .3003 gram uric acid. Of all the alkalis used, sodium carbonate seemed to be the least destructive, but it was found utterly impossible to dissolve the acid to the extent stated by Burian, who used only twenty cubic centimeters of a half normal solution of sodium carbonate with twenty cubic centimeters hot water to three hundred and sixty cubic centimeters of the organ extract for .5 gram uric acid. This method was closely followed by me, but a portion of the acid always fell out of solution and I was forced to have recourse to stronger solutions of this and other alkalis. Next apparently comes disodium phosphate, then lithium carbonate; while last and most destructive of all is sodium hydrate, which, though used in the same strength as Shittenhelm used it of only .024 gram to four hundred cubic centimeters fluid, has a most energetic action on uric acid, and attention is called once more to the apparent indifference with which the above author passes over the loss of one-sixth of his uric acid. If as apparent this acid is so readily broken up by alkali, how can it persist in the blood, for in several instances where the loss was marked, the alkalinity of these mixtures as shown by titration with N/25, tartaric acid is not as great as that of the blood under similar circumstances. For instance, in nine the alkalinity determined in this way was equal to only .073 gram NaOH in one hundred cubic centimeters, while in ten it was only .096 in one hundred cubic centimeters. The effect of lithium carbonate is equally as well marked, nor is there any improvement but the reverse when sodium sulphide is used for the removal of the silver, as would be expected. In spite

of the great rapidity with which filtration was accomplished the deleterious effect of the sodium sulphide can be seen; as can also be seen from the nitrogen determinations the isolated uric acid was somewhat contaminated, since the amounts determined by weighing and determination from nitrogen do not exactly correspond.

Enzymatic action of organ extracts on uric acid. — Since disodium phosphate has apparently the least destructive effect upon uric acid of the alkalis used, this was employed as a solvent. The organs which were extracted for an enzyme were ox spleen and liver, and a human liver obtained twenty-four hours after the death of the victim from accident. Owing to the difficulty of obtaining a solution entirely free from albumin by boiling the digestive mixture with acetic acid and sodium chloride, the method of V. Schröder⁶ was followed, since the silver forms a colloid substance with any albumin present, which is very difficult to decompose with hydrogen sulphide. In every case ample protection against bacterial action was afforded by saturating the mixture with chloroform and then pouring in toluol until it formed a layer over the surface of the fluid. The results of these experiments can be seen in Table II.

TABLE II.

No. of Experiment.	Source of Enzyme.	Nature and Amount of Alkali.	Period of Digestion.	Uric Acid Recovered (by Weight).	Uric Acid Recovered (from N).	Per Cent Acid Destroyed.	Remarks.
17 A	Ox spleen.	9 g. Na ₂ HPO ₄ .	114.5 hrs. on ice; cooked ¼ hour.252 g.	16	Isolated by (NH ₄) ₂ SO ₄ and dialysis.
B	" "	" "	114.5 hrs. at 38°.258 g.	14	
C	" "	" "	" " "079 g.	73.6	
18 A	Ox liver.	10 g. Na ₂ HPO ₄ .	6 days on ice; cooked ¼ hour.	.1486 g.	50.8	Isolated by Rosell method 2 hours, 40 minutes after removal of organ.
B	" "	" "	6 days at 38°.	.0226 g.	79.1	
C	" "	" "	" " "	.0853 g.	71.5	
19 A	Ox liver.	No alkali.	5 days on ice; cooked ¼ hour.	.181 g.	39.6	Isolated by Rosell method 2 hours, 40 minutes after removal of organ.
B	" "	" "	5 days at 38°.	.176 g.	41.3	
C	" "	" "	" " "	.146 g.	51.3	
20 A	Ox liver.	4 g. Na ₂ HPO ₄ .	48 hrs. on ice; cooked ¼ hour.1106 g.	62.1	Isolated by Rosell method 24 hours after death.
B	" "	" "	48 hrs. at 38°.0666 g.	77.8	
C	" "	" "	" " "1176 g.	62.8	
21 A	Human liver.	5 g. Na ₂ HPO ₄ .	71 hrs. on ice; cooked ¼ hour.	Lost.	Isolated by Rosell method 24 hours after death.
B	" "	" "	71 hrs. at 38°.2547	
C	" "	" "	" " "3036	

* Uric acid added after cooking.

The amount of organ extract used in every case was four hundred cubic centimeters and uric acid .3 gram. The silver was removed by H₂S.

It may be said that, in every instance, great care was taken that an excess of silver was added and this fact always verified by making a portion of the filtrate acid by the addition of nitric acid by which the silver chloride is rendered insoluble. Great care was taken also that the solution should be at least neutral before it was cooked to remove the albumin present. Upon examining the results we note at once that with the exception of ox spleen there is no marked evidence that a uric acid splitting enzyme is present. Further, the marked effect of the alkali is seen at once. Even in those portions which were kept on ice there is evidence that alkali was active, while in the portion which was cooked the activity of the alkali is distinctly seen, since in these the greatest loss occurs, instead of in C where the greatest loss should have occurred, were there an efficient enzyme present. In the case of human liver, a slight destruction above what is clearly due to the alkali is seen. In nineteen, where no alkali at all was used, the results of isolation are much better, though there is apparently but little destruction, which may be attributed to a possible enzyme. Further, in this case, there must be a loss from inability to separate the acid from the albumin coagulum, on account of its slight solubility in water. On the whole we may say that evidences of enzymatic action are shown only in the extract from the ox spleen.

Products of the splitting of uric acid. — In my last communication attention was called to the fact that oxalic acid and allantöin were both found as a product of splitting, of which the allantöin was too small in amount to be verified other than by its microscopic appearance, which was compared with a preparation obtained by the action of potassium permanganate on pure uric acid; these products were very similar in the form of their crystals and in their arrangement, and photomicrographs were obtained of them for future reference. In this series of experiments urea was also sought for on account of the ready union of glyoxylic acid and urea to form allantöin, from the former of which oxalic acid may

be readily formed. As is well known, uric acid may be converted by oxidation to urea and oxalic acid through alloxan and parabanic acid. Hence there are two possibilities in the splitting of uric acid, in both of which the final products are oxalic acid and urea. The method employed was as follows: The filtrate left from the precipitate of uric acid was acidified strongly with hydrochloric acid and repeatedly extracted with ether, the ethereal extracts treated according to Salkowski⁷ for oxalic acid; the remaining watery fluid was evaporated to dryness and extracted with absolute alcohol, which will dissolve the urea but not the allantöin, and the usual tests applied to the residue from the alcoholic extract. The residue after extraction by alcohol was dissolved in a little water, filtered and treated as described in my former communication. In eight cases where the splitting was produced by alkali and warmth, oxalic acid was found in five and absent in three, while urea and allantöin were not found at all. In six digestions where the uric acid was subjected to an organic extract and alkali, oxalic acid was found in three cases and absent in three, while urea and allantöin were not found at all.

We cannot exclude the possibility that this splitting was due to the alkali alone since in the third digestion, where every opportunity was given for the most energetic action of the supposed enzyme, oxalic acid did not appear more frequently than in the first and second, where cold in the first and cooking in the second should have destroyed the enzymatic action. It is quite apparent, however, that oxalic acid is a fairly common product of the splitting of uric acid by alkali. Whether this is through alloxan and parabanic acid or through allantöin cannot as yet be determined, for if allantöin is formed as appears probable from its occasional appearance, then it must be further broken up and form oxalic acid.

Reversibility of enzyme. — Many of the known enzymes have a reversible action, in other words, put together the substances which they have split from a more complex

substance, to form the latter. We need only mention in illustration maltase, which can split maltose into two molecules of dextrose and again unite the latter to form maltose. Therefore in order to demonstrate further the actual existence or non-existence of uricolytic enzyme, attempts were made to unite various substances which either by chemical means or by biological action have been made to form uric acid or have been split off from it. Such substances according to Wiener⁸ are lactic acid, oxalic acid, and glycerine. An extract was prepared from ox liver according to the method of Rosell mentioned above, and in this in definite quantities these three substances were brought singly with urea well protected by chloroform and toluol and placed in the brood oven. At the same time a control was made of the extract alone, because purin bases may be present in such extracts which may be readily converted to uric acid. The results of these experiments will also be found arranged in the table which follows:

TABLE III.

No. of Experiment.	Amount Urea.	Amount Lactic Acid.	Amount Oxalic Acid.	Amount Glycerine.	Time of Digestion.	Uric Acid Recovered (by Weight.)	Uric Acid Recovered (from N).	Remarks.
22 A.....	0	0	0	0	4 days at 38°.0498 g.	Ag. removed by H ₂ S.
B.....	1.2 g.	0	1.26 g.	0	" " "052 g.	
C.....	1.2 g.	.9 g.	0	0	" " "0526 g.	
D.....	1.2 g.	0	0	1 g.	" " "043 g.	
23 A.....	1.2 g.	0	1.26 g.	0	5 days at 38°.	.0534 g.	.0486 g.	
B.....	1.2 g.	.9 g.	0	0	" " "	.0594 g.	.0573 g.	
C.....	1.2 g.	0	0	1 g.	" " "	.0499 g.	.0528 g.	

In every case the amount of liver extract used was four hundred cubic centimeters.

It can be plainly seen that the control to which nothing was added contains approximately the same amount of uric acid as those portions of the liver extract to which the various substances were added; the difference is so small that it may be readily attributed to the unavoidable inaccuracies of the process of determination. That the liver extract itself contains so much uric acid is due to the conversion of the nuclein and xanthin bases to uric acid as shown by Schittenhelm⁹ and also by Jones and Austrian.¹⁰ Hence we may say without hesitation that there is no evidence of a reversible action of any enzyme which may be present. In order to determine whether possibly any allantoin was formed, all of the filtrates from which the uric acid had been removed were carefully examined for that substance but always in vain.

Briefly we may sum up the results of this investigation as follows: All alkalis, even the weaker ones, at brood oven temperature split uric acid, an action which almost invariably increased as the temperature rises. The destructive action of the alkali used for the solution of uric acid is not uniformly increased by the addition of organic extracts isolated by the method of Rosell, which is supposed to isolate an active enzyme if such exists. Among the products of splitting by both the alkali and the alkali in conjunction with organic extracts, oxalic acid is quite uniformly found, urea and allantoin not at all. It is very difficult to isolate an extract of organs which does not contain purin bases, which are readily converted to uric acid at brood oven temperature by xanthooxydase, thereby obscuring the results obtained by isolation of the remaining acid. There is no evidence of the reversible action of the uricolytic enzyme if such exists. Unless some solvent other than an alkali is found for uric acid, it will be difficult to separate the destructive action of the alkali from that of any enzyme which may be present.

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B. COLI COMMUNIS, "THE PRESUMPTIVE TEST," AND THE
SEWAGE STREPTOCOCCI IN DRINKING WATER.*

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That the presence of *B. coli communis* in a water is generally recognized as an indication of sewage pollution needs no further comment here. That the absence of it, however, indicates the purity of a water is a subject, I believe, worthy of consideration. The question arises: Should we consider a water free from pollution in which no *B. coli* is found? Further, is the presence or absence of *B. coli* always indicated by the "presumptive test"? Finally, what is the indication of the "Sewage streptococcus"? These questions gave rise to the investigation upon which this paper is based. It embraces observations covering two years upon the water supply of the city of Philadelphia, and comprises a series of comparative tests made with the raw water of the Schuylkill river, after sedimentation or preliminary rough filtration, as well as after purification by slow sand filtration.

In a previous paper I pointed out some observations regarding the Colon group in drinking water, and I believe that the technic usually employed for its detection is subject to such a difference of opinion, individual preferences of each bacteriologist in some cases, and dogmatic statements in others, that it is a question whether we are justified in expressing an opinion based upon the researches of this group alone. Details are not necessary at this point, but I desire to emphasize that we are dealing with atypical forms of *B. coli* in some cases when we only consider the bacteriological analysis of water, and moreover, since the technic employed is very limited in extent, the discovery of the colon bacilli alone should not always be considered sufficient indication of the pollution of water. This statement, I believe, will be admitted by at least some bacteriologists.

Based upon the presumption that *B. coli* is an associate of

*Received for publication Jan. 21, 1907.

B. typhosus, and that it is a common inhabitant of the intestine, its presence in a water is considered as an indication of pollution. It being well known, however, that this micro-organism is not found in the human intestines only, but is widely distributed in nature. Inasmuch as it is supposed to gradually disappear from water, it has been suggested that we base our judgment not merely upon the qualitative but more especially the quantitative estimation: that is, the number of *B. coli* found should be given more consideration than its mere presence or absence. In view of the foregoing, are we always sure to obtain harmonious results in two bacteriological analyses made at the same time?

Experiments with sterile water artificially inoculated with *B. coli* will naturally give the same result, while previously contaminated water artificially inoculated with *B. coli* will only exceptionally give harmonious results. As this is exactly what happens in nature, any bacteriologist, familiar with the examination of water, undoubtedly has come across such discrepancies. The irregularity of the results, met in the following experiments, suggested to the writer their publication, in the hope that they may demonstrate the limited value which in some cases the search for the *B. coli* presents. It is not my purpose to declare such examination useless, but in spite of the valuable results obtained, to ask: Can we at present say what, after all, is the number of *B. coli* per cubic centimeter of a water which may be regarded as safe, suspicious or dangerous? Furthermore, given a water in which no *B. coli* are found, can it be regarded as safe? As neither one of these questions (some bacteriologists will admit, and I hope to prove by the following results) can be satisfactorily answered, is sufficient evidence. I believe that in the search for the Colon group, we are dealing more with the symptoms than with the cause of pollution in drinking water.

The bacteriological technic usually employed for the study of the Colon group consists in the plating of the water in Wurtz litmus-agar plates, to which one and a half to two per cent of Parietti's solution has been added, examination of the

plates after twenty-four hours at 37° C., selection of the characteristic pink colonies resembling *B. coli*, and further study of the same for the fermentation of sugar, production of indol, reduction of nitrate to nitrite, non-liquefaction of gelatine, and other biological features of the Colon group; to these usually the studies are limited. The morphological, and in general the microscopical researches, are neglected, not because they are not so important, but because they are tedious and require time. Further, the search for the sewage streptococci, claimed to be an indication of pollution in water, is almost entirely neglected in everyday work; and for the same reason, limited time, some bacteriologists confine themselves merely to the "presumptive test," the shortest of all researches for the Colon group. The important question to be settled is, which is the most reliable of the indications? The mere fact that there is a number of them is sufficient reason to assume that while there is some reliance to be placed on each, none is sufficiently accurate in itself.

In the present work no time has been spared or labor omitted. It has been my purpose to give to each test the most exhaustive trial, and to present to the best of my ability the results obtained, leaving the reader to draw his own conclusions. The general counting of bacteria and number of *B. coli communis* per cubic centimeter, the study of the sewage streptococci and the "presumptive test" on the same water, are considered at the same time, and independently, and I believe that though valuable researches have been made upon each subject separately, the combined observation of them here presented will aid toward a better understanding of the results.

The raw water represents the water from the Schuylkill river at different places, Belmont pumping station (for the supply of Belmont) and Shawmont (for upper and lower Roxborough reservoir). Such water is found to be of a considerable degree of pollution, containing in only a few cases below 10,000 and in some instances over 200,000 bacteria per cubic centimeter. In other cases there were found over

500,000 bacteria per cubic centimeter. The applied water corresponds to the same water after sedimentation in the case of Belmont and upper Roxborough reservoir, and after preliminary rough filtration for the lower Roxborough filters. Contrary to what would be expected, an irregularity is observed in some cases in which the applied water showed an equal or higher number of bacteria per cubic centimeter. Often the effect of rains persisted longer in the reservoir and applied water than in the river, where the water is constantly changing, and clears more quickly, with consequent diminution in the number of bacteria. This, however, should not detract from the results, as each water was examined separately on the same day. Finally the effluent water is compared with the applied water after purification by slow sand filtration. Here again some countings are relatively high, due to recent removal of the "schmutzdecke."

As will be observed in the tables, the first line on each date corresponds to the Belmont, and the second, to the upper and lower Roxborough plant, respectively.

It is not necessary to give in detail the technic employed in the search for the Colon group, a description of which was given in a previous paper, and has been briefly outlined above. It suffices to say that all the biological and morphological characters of *B. coli* as well as its variations were considered. For the sake of brevity, the technic employed for "the presumptive test" is also omitted, a description of which can be found in any text-book on bacteriology, or manual of water analysis. Atypical reaction marked + ? on the tables indicates results in which the inoculation of one cubic centimeter of the water on dextrose broth gave an amount of gas after forty-eight hours at 37-40° C. above ninety per cent or below ten per cent.

The search for the sewage streptococci consists in the inoculation of one cubic centimeter of the water in dextrose broth and daily microscopical examination of the culture up to six days at 37-40° C. according to the case; by this procedure, it was possible to obtain positive results with cultures which in the first few days failed to show any cocci. With this preliminary technic, the results obtained are as follows:

Date.	Raw Water.				Applied Water.				Effluent Water.			
	Bacteria per cc.	B. coli per cc.	Pre- sumpt. Test.	Sewage Strept.	Bacteria per cc.	B. coli per cc.	Pre- sumpt. Test.	Sewage Strept.	Bacteria per cc.	B. coli in		Pre- sumpt. Test.
										1 cc.	50 cc.	
Nov. 20 . . .	16,000	0	+	0	3,400	0	+	0	78	0	+	+
" "	18,000	0	+	0	340 14,000	0 1	+	0	28 14	0 0	0 0	0 +
Nov. 21 . . .	12,000	—	—	—	15,000	2	+	0	45	0	0	0
" "	48,000	15	+	0	1,200 11,000	1 1	+	0	32 160	0 0	0 0	0 0
Nov. 22 . . .	18,000	—	—	—	11,000	1	+	0	43	0	0	0
" "	20,000	4	+	0	12,000 6,500	0 0	+	+	32 90	0 0	0 0	0 0
Nov. 27 . . .	36,000	5	+	0	18,000	0	+	+	8	0	0	+
" "	39,000	2	+	+	1,800 22,000	0 1	+	0	12 119	0 0	0 0	0 0
Nov. 28 . . .	44,000	—	—	—	20,000	10	+	0	22	0	+	0
" "	60,000	20	+	0	3,800 15,000	0 5	+	0	16 45	0 0	0 0	0 0
Nov. 29 . . .	28,000	—	—	—	14,000	1	+	+	14	0	0	+
" "	48,000	0	+	0	4,200 8,000	1 0	+	0	27 28	0 0	0 0	0 0
Dec. 4	120,000	27	+	+	78,000	7	+	+	240	0	+	+
" "	1,120,000	33	+	+	20,000 21,000	1 4	+	+	130 220	0 0	0 0	+

Date.	Raw Water.				Applied Water.				Effluent Water.				
	Bacteria per cc.	B. coll per cc.	Pre sumpt. 'feet.	Sewage bioppt.	Bacteria per cc.	B. coll per cc.	Pre sumpt. 'feet.	Sewage Strept.	Bacteria per cc.	B. coll in 1 cc.	B. coll in 50 cc.	Pre sumpt. 'feet.	Sewage Strept.
Dec. 5, . . .	68,000	-	-	-	51,000	16	-	+	120	0	0	+	+
" " " " "	120,000	27	+	+	100,000 18,000	3 10	+	+	170 170	0 0	+	0	+
Dec. 6, . . .	110,000	-	-	-	110,000	20	+	+	200	0	+	+	0
" " " " "	210,000	40	+	+	14,000 25,000	3 20	+	+	95 1,160	0 0	0	+	0
Dec. 11, . . .	60,000	7	+	+	23,000	10	+	+	110	0	0	+	0
" " " " "	35,000	20	+	+	7,000 4,500	2 10	+	0	140 140	0 0	0	+	0
Dec. 12, . . .	10,000	-	-	-	20,000	10	+	0	71	2	0	0	+
" " " " "	10,000	0	+	+	7,500 4,700	8 4	+	+	110 120	0 0	0	+	0
Dec. 13, . . .	6,000	-	-	-	20,000	7	+	0	85	0	+	+	0
" " " " "	73,000	0	+	0	45,000 6,000	4 3	+	0	50 80	1 2	0	+	0
Dec. 15, . . .	14,000	0	+	+	23,000	1	+	+	66	0	0	+	0
" " " " "	53,000	1	+	0	8,000 14,000	0 1	+	0	50 46	0 0	0	+	0
Dec. 20, . . .	65,000	-	-	-	28,000	0	+	0	68	0	0	0	0
" " " " "	3,000	0	+	0	13,000 6,100	0 1	+	0	41 34	0 0	0	0	0

B. COLI COMMUNIS.

Dec. 26	24,000	-	-	-	48,000	14	+	+	+	240	0	0	+	+
" "	19,000	14	+	+	{ 12,000 16,000	2 14	+	+	+	36 23	0 0	0 0	0 +	+
Dec. 27	19,000	-	-	-	32,000	10	+	+	+	260	0	0	0	+
" "	16,000	14	+	+	{ 10,000 14,000	1 4	+	+	+	31 29	0 0	0 0	0 +	+
Dec. 28	24,000	-	-	-	39,000	3	+	+	+	30	0	0	0	0
" "	37,000	6	+	+	{ 31,000 24,000	2 4	+	+	+	21 50	0 0	0 0	0 0	0
Jan. 31	24,000	-	-	-	12,000	4	+	+	+	14	0	0	+	+
" "	44,000	20	+	+	{ 4,000 28,000	0 1	+	+	+	36 35	1 0	0 0	+	+
Feb. 5	180,000	1	+	+	52,000	2	+	+	+	8	0	0	0	+
" "	70,000	1	+	+	{ 4,000 30,000	2 0	+	+	+	45 42	0 0	0 0	0 0	0
Feb. 6	51,000	-	-	-	12,000	1	+	+	+	10	0	0	0	0
" "	62,000	2	+	+	{ 9,500 44,000	0 0	+	+	+	48 28	0 0	0 0	0 0	0

In a consideration of the results, contrary to what should be expected, it is noted that there are some cases in which the raw water did not show any *B. coli*, while in the corresponding applied water, this organism was found in company with a very low bacterial count. Such discrepancy is not only observed between the raw and applied water, but also in the effluent water with a very low number of bacteria, after purification by slow sand filtration. Further it is observed that in the applied water, in spite of the lower number of bacteria in general, *B. coli* was found in greater number than in the corresponding raw water. The same can be said of the limited value which the research on more than one cubic centimeter presents, a glance at the tables showing the cases in which *B. coli* was found in one cubic centimeter of the effluent water while none was found in the fifty cubic centimeter tests.

"The presumptive test," regardless of the quality of the water, and the presence or absence of *B. coli*, was positive in all the raw and applied water but one sample, and similar results were obtained with the effluent water, but the most marked irregularity was observed in the search for the sewage streptococci. There are cases in which both the raw and applied water failed to show any, while in the effluent water with a general count below fifty and even as low as eight bacteria per cubic centimeter, this microörganism was observed; often in the high counts no streptococci were found. In view of the fact that this irregularity could be attributed to the imperfect technic employed, as in the incubation of one cubic centimeter of the water, other microorganisms were also inoculated which could bring about a symbiotic process detrimental to the sewage streptococci. To avoid this, it was desirable to make some modification, and this change consisted in the plating of one cubic centimeter of the water in litmus-lactose-agar, and the study of the characteristic colonies after twenty-four to forty-eight hours at 37-40° C.

This is not the place to consider the ten to twenty different types of sewage streptococci described in the literature;

from the pink colonies on litmus-lactose-agar to no change of the medium and even blue colonies; from very small and almost imperceptible fine points to relatively large colonies; from the rapid, to slow and non-liquefiers of gelatine; coagulation and non-coagulation of milk; production and non-production of indol; strongly acid reaction to neutral or even alkalinity of the medium. Other biological characters were considered, and great variation was observed in the morphology, some appearing as diplococci or resembling staphylococci, others intermediate between these and streptococci, while still others were typical streptococci. Some appeared in short, while others in very long chains. The individual organisms were very small in some cases, and in others relatively large. The Gram stain was taken by all cultures.

Without going into details of the variations met in water from different sources and the number of sewage streptococci per cubic centimeter determined by the plate method, a description of which I regard as unessential to the purpose of this paper, it suffices to say that the results reported comprise only the cases in which more or less an approach to the sewage streptococcus types were obtained.

Besides the plate methods, the inoculation of one cubic centimeter in dextrose broth was also considered independently as before. The results are as follows:

Date.	Raw Water.			Applied Water.			Effluent Water.			
	Bacteria per cc.	B. coli per cc.	Pre-sumpt. Test.	Sewage Strept.	Bacteria per cc.	B. coli per cc.	Pre-sumpt. Test.	Sewage Strept.	B. coli in	
									1 cc.	50 cc.
Feb. 13. . . .	150,000	—	—	—	490,000	0	+	0	0	+
" "	170,000	20	+	0	86,000	0	+	0	0	+
Feb. 14. . . .	160,000	—	—	—	550,000	1	+	0	0	+
" "	160,000	5	+	+	160,000	0	+	0	0	+
Feb. 19. . . .	100,000	20	+	+	52,000	0	+	0	0	+
" "	200,000	27	+	+	78,000	4	+	0	0	+
Feb. 21. . . .	60,000	—	—	—	170,000	13	+	0	0	+
" "	160,000	37	+	+	64,000	3	+	0	0	+
Feb. 26. . . .	39,000	33	+	+	120,000	8	+	0	+	+
" "	64,000	40	+	+	36,000	0	+	0	+	+
Feb. 27. . . .	70,000	—	—	—	94,000	2	+	0	+	+
" "	64,000	40	+	+	69,000	40	+	2	0	+
Feb. 28. . . .	41,000	—	—	—	4,200	1	+	0	0	+
" "	41,000	100	+	+	25,000	0	+	0	0	+
" "	41,000	—	—	—	57,000	27	+	0	0	+
" "	41,000	—	—	—	33,000	0	+	0	0	+
" "	41,000	—	—	—	42,000	2	+	1	0	+
" "	41,000	—	—	—	28,000	0	+	1	0	+
" "	41,000	0	+	+	2,400	0	+	0	0	+
" "	41,000	—	—	—	26,000	20	+	0	0	+

	60,000	100	+ —	+ —	+ —	47,000	100	+ —	+ —	+ —	160
March 5 . . .											
" " . . .	100,000	120	+	+	+	{ 4,400 50,000	0 50	+	++	+	{ 46 68
March 6 . . .	25,000	—	—	—	—	30,000	5	+	+	+	420
" " . . .	24,000	50	+	+	+	{ 5,600 35,000	1 2	+	++	+	{ 48 80
March 7 . . .	7,800	—	—	—	—	39,000	20	+	+	+	270
" " . . .	11,000	30	+	+	+	{ 7,500 27,000	2 10	+	++	+	{ 70 92
March 12 . . .	18,000	20	+	+	+	5700	0	+	+	+	33
" " . . .	14,000	30	+	+	+	{ 2,800 5,800	1 7	+	++	+	{ 63 44
March 13 . . .	19,000	—	—	—	—	5700	2	+	+	+	19
" " . . .	26,000	20	+	+	+	{ 2,400 7,000	0 0	+	++	+	{ 38 30
March 14 . . .	15,000	—	—	—	—	11,000	3	+	+	+	22
" " . . .	10,000	12	+	+	+	{ 700 6,600	0 1	+	++	+	{ 50 50
March 19 . . .	24,000	0	+	+	+	11,000	1	+	+	+	45
" " . . .	32,000	1	+	+	+	{ 1,200 2,400	0 0	+	++	+	{ 43 50
March 20 . . .	14,000	—	—	—	—	7,400	2	+	+	+	24
" " . . .	120,000	30	+	+	+	{ 2,700 3,600	2 2	+	++	+	{ 26 44
March 21 . . .	150,000	—	—	—	—	16,000	1	+	+	+	38
" " . . .	19,000	10	+	+	+	{ 3,200 6,800	2 2	+	++	+	{ 60 74

Date.	Raw Water.				Applied Water.				Effluent Water.			
	Bacteria per cc.	B. coli per cc.	Pre- sumpt. Test.	Sewage Strept.	Bacteria per cc.	B. coli per cc.	Pre- sumpt. Test.	Sewage Strept.	B. coli in		Pre- sumpt. Test.	Sewage Strept.
									1. cc.	50 cc.		
March 26 . . .	36,000	5	+	0	50,000	0	+	0	26	0	0	0
" " . . .	57,000	0	+	0	3,100 27,000	0	0	0	53 40	0	0	0
March 27 . . .	14,000	—	—	—	48,000	1	+	0	35	0	0	0
" " . . .	170,000	30	+	+	2,600 13,000	0	+	+	38 98	0	0	0
March 28 . . .	64,000	—	—	—	8,000	2	+	+	14	0	0	+
" " . . .	91,000	14	+	+	3,400 51,000	0	0	+	52 58	0	0	0

Concerning *B. coli*, the results show the same irregularity as before, though less markedly. For the sake of brevity, I deem it unnecessary to repeat what has been considered above, and it suffices to add that the number of *B. coli* per cubic centimeter was not always in proportion to the source and quality of the water examined ; further there are cases here in which samples of water with over 400,000 and 500,000 bacteria per cubic centimeter show in one case none, and in the other one of these microörganisms, while in the corresponding raw water, with a decidedly lower number of bacteria, twenty *B. coli* per cubic centimeter were found.

Similar irregularity is observed in "the presumptive test," and there are samples of water with high bacterial count containing *B. coli* in which this test was negative and vice versa.

Regarding the sewage streptococcus, the improvement of the plate method for its detection brought about a decided increase of positive results, but regardless of the source and quality of the water, the presence or absence of this micro-organism did not seem to have any special importance. The statement that its presence is an indication of old sewage contamination and that it is more apt to be found in cases where *B. coli* has disappeared from the water does not seem to be in accord with the results here reported, as it was found associated with this group as well as in the absence of it. Moreover, I believe the streptococcus has little weight in the conclusion as to the purity of water.

Before closing, I desire to emphasize the fact that it has not been the purpose of this article to regard the search for the Colon group as unimportant, nor to condemn the technic which each bacteriologist may prefer for its detection, but merely to express some view upon the results above reported.

CONCLUSIONS.

(1.) The search for *B. coli communis* undoubtedly has its merit, but I believe its value to be limited, and that too much reliance is placed upon this group of organisms ; further, the slight difficulty which its detection presents is

responsible for the entire neglect of the search for other pathogenic bacteria, *B. typhosus* especially, which though not so easily detected is of such importance as to stimulate an improvement in our present routine in bacteriological water analysis.

(2.) That the "presumptive test" alone is insufficient and uncertain in diagnostic value for *B. coli*.

(3.) The presence of the sewage streptococcus is of little if any importance, and bears the same relation in some cases as many other harmless water bacteria commonly found.

[In conclusion I wish to express my appreciation of the courtesy extended to me by Dr. Samuel G. Dixon, Commissioner of Health, of the State of Pennsylvania, for his valuable criticisms on this paper, and to Dr. Bergey, Assistant Professor of Bacteriology at the University of Pennsylvania, for some suggestions in this work.]

LESIONS OF THE GRANULE LAYER OF THE HUMAN CEREBELLUM.*

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The granule layer of the cerebellum is peculiar in construction. The layer is very rich in cells, surpassing most regions of the nervous system in the proportion of cells to the unit of space. Very noteworthy, in comparison with the general cell wealth, is the relative paucity in this locus of interstitial substance. Barring its vessels, the layer is, so to speak, almost purely nervous in structure. A further peculiarity of the granule layer is one shared by adjacent layers, viz., an almost uniform character throughout the cerebellum. The several cell types, the fibers, and the characteristic acidophile structures of Denissenko form altogether a most complex-looking apparatus; but the complexity is a uniform complexity throughout the organ.

The most striking feature of the granule layer is doubtless the relative wealth of cells therein. There are few fixed tissues, barring tissues concerned with continuous generation of cells, elsewhere in the body which present so many nuclei to the unit of space. In the central nervous system itself, there are few loci which approach the granule layer in relative nuclear richness, excepting some parts of the limbic lobe and the stellate cell layers of the cerebral (particularly the calcarine) cortex. There is no good evidence of multiplication in the nerve elements of the granule layer, at any rate after early life.

Despite this general cell-wealth, the granule layer is notably lacking in neuroglia tissue, that is to say, in cells producing neuroglia fibrils, under normal conditions. This fact was observed by Weigert in 1895. The methods of Mallory

* Received for publication Jan. 25, 1907.

Read at the annual meeting of the American Association of Pathologists and Bacteriologists, Baltimore, May, 1906.

and of Benda go far to confirm the point. This lack of neuroglia tissue in the granule layer is the more surprising when contrasted with the presence of considerable fibrillar substance, as demonstrated by the above methods, in adjacent layers. Thus the underlying white matter and the overlying Purkinje cell belt are well supplied with neuroglia fibrils, while the molecular layer still finds space among the branches of the Purkinje cell dendrites for a number of fibrils, even under normal conditions. The granule layer of the cerebellum may be logically grouped, therefore, with various peripheral nerve structures in the vertebrates, in its lack of neuroglia. This lack, however, is readily supplied under abnormal conditions.

Besides the relative cell wealth and (perhaps also relative) paucity of neuroglia tissue, the granule layer also shows a strikingly constant arrangement of the constituent elements, which holds not only throughout a given lamina but also, so far as known, throughout the cerebellar cortex. The cell arrangements, not merely of the granule layer, but also of the exterior layers, in the cerebellum are far more nearly uniform (or less differentiated ?) than the cell arrangements of the cerebral cortex. There is good ground for calling the cerebral cortex a composite of organs. So far, however, the cerebellum seems as uniform as it is complex. This fact has given rise from early days to the idea that the cerebellum acts as a unit. In any case, the granule layer is obviously a structural unit in the cerebellum.

What part shall be assigned to the granule layer in cerebellar activity? We might argue something of its functions from its situation between the core of white matter and the Purkinje cell belt. The presence of incoming and outgoing nerve fibers in the core of white matter obviously suggests that the activities of the cerebellar cortex complete an arc of impulses which flow in those fibers. It seems well established that the Purkinje cells are the locus of immediate origin for outgoing impulses. In case this is so, then the granule layer may probably serve, as seems likely from its site, as part of the centripetal mechanism.

As an example of the difficulties lodging herein, the hypothetical account of Kölliker, based on the work of Ramón y Cajal and others, may be cited. Kölliker (1896) regarded the Purkinje cells as the centrifugal elements, the moss fibers and climbing fibers (of the Golgi pictures) as the centripetal elements of the cerebellar cortex. Counting the Purkinje cells as the only centrifugal cells, it becomes hard to interrelate the remainder of the cells, classified by Kölliker as large and small granule cells, basket cells, and small cortical cells, which must all be related with the centripetal apparatus. Kölliker proposes that the moss fibers shall come into relation with the small and large granule cells, that the large granule cells shall be association cells, and that the small granule cells shall by means of their processes in the molecular layer come into relation with the Purkinje cell dendrites. This account leaves outstanding the basket cells and the small nerve cells of the cortex. Kölliker proposes that the climbing fibers and other centripetal fibers shall come into relation with these latter cells and these in turn with the Purkinje cell dendrites once more.

This account of Kölliker's yields some notion of the number of elements with which we deal. The account leaves out, moreover, the acidophile structures of Denissenko, which, as shown below, are remarkably resistant in disease.

Not to consider minutiae at this point, it is evident that a true solution of these and kindred problems might be attained if we could differentially destroy the various elements in turn and watch the effect upon the activities of the apparatus in lower animals. A slower but perhaps surer method of attack might be to watch the effect of such differential or uncomplicated destruction of the various elements in man in the course of disease.

In default of a more rapid method of work, I have thought it profitable to look through a considerable number of cases, both with and without gross signs of cerebellar disease, to find what are the common lesions of the granule layer. An ulterior object was to arrange the elements of the granule layer in a series according to their resistance to injurious agents.

The present work is related to some previous work printed in 1905, where I described in some detail the characteristic lesions of the exterior layers in cases of marginal sclerosis in the human cerebellum. To these lesions there proved to be a rather obvious natural history. Thus the early lesions found in leptomeningitis (particularly tuberculous) could be aligned with the later lesions in local ischemia (well shown in infarctions of luetic origin). The series of changes thus made clear were used to explain the peculiar appearances — fibrillar overgrowth in three planes — in cases of stationary or terminal fibrillar gliosis. The first stage of this fibrillar gliosis in three planes was shown to be an overgrowth of the characteristic radial fibrils (Bergmann's fibers) of the molecular layer, an overgrowth frequently related with the death of Purkinje cells. The cells of origin for these radially shooting fibrils were found to be certain neuroglia cells in the Purkinje cell belt. In accord with the established conception of the Purkinje cells as the cells of least resistance in the cerebellum, it was found that the Purkinje cell belt is the locus of earliest activity in repair.

At present I wish to consider in a similar way the natural order of lesions in the inner (granule) layer of the human cerebellum. I have presented the cases on which the points are based in summary form. I have used various special methods on the material, but have depended for the general analysis of the findings in the granule layer upon various methods after fixation in Zenker's fluid, especially upon eosin and methylene blue, Mallory's aniline blue, and Mallory's phosphotungstic acid hematein methods, used on paraffine sections.

CASE I. Case of defective girl, born without the use of instruments in the eighth month, one of twins (the other still-born). Slight evidence of consciousness or orientation (even for mother). General rigidity. Food given with difficulty. Constipation. Lived in cradle on its back. Convulsive seizures began after twentieth month. Death in thirty-seventh month from bronchopneumonia.*

* For clinical history in full, see Bullard and Southard. A case of idiocy in a child with cystic hemispheres. Medical and Surgical Reports, Boston City Hospital, XV., 1905.

The autopsy showed gross cerebral changes. The ground plan of the cerebrum was preserved, but the substance of each hemisphere was largely replaced by a closed cystic cavity, independent of the ventricles. The interiors of the cystic cavities were traversed by delicate strands of neuroglia tissue. The pyramidal tracts had failed to develop.*

The cerebellum showed no reduction in size and possessed the usual markings.

The microscopic examination of the cerebellum in this case showed that the granule layer was far richer in cells, relatively to the unit of space, than the granule layer of the adult (see Plate IV., Figs. 1 and 2). The small cells called granule cells possess somewhat more vesicular nuclei than similar cells in the adult and are more closely packed together. The clear spaces between granule cell groups are more sharply marked off than in the adult and contain in some cases the characteristic acidophile structures of Denis-senko. In other cases, however, the clear interspaces contain a nucleated structure in place of the non-nucleated structure of Denissenko. These structures, not seen in the adult, are spread not alone through the granule layer but also through the core of white fibers (where also they fail to occur in the adult). These cells have large homogeneous cell-bodies, rounded or oval in shape, and vary in size to above 60μ . They are supplied with single or sometimes with two rather dense central nuclei (see Plate IV., Fig. 3). There is no evidence from the tissues of this case that these cells produce neuroglia fibrils. It is still probable that they are cells of the interstitial or neuroglia series. It is not improbable that these cells in the granule layer may give rise to the acidophile structures of Denissenko. Of this, there is no proof, save that of suggestive situation and certain apparent transitions.

The case brings out the relatively even greater cell-wealth of the granule layer early in development and the occurrence or persistence of certain cells, probably neuroglia cells, which may be related with the development of the acidophile

* For photographs showing cerebral aplasia of this case, see Bullard and Southard. Cystic aplasia of the cerebral hemispheres in an idiot child. Journ. Med. Research, XIV., 2, January, 1905.

structures of Denissenko. The cell arrangements throughout the cerebellar cortex are uniform.

CASE II. Epileptic imbecile of sixty-five years. One brother imbecile. Learned to walk at four or five. Never learned to speak beyond "yes" and "no," "papa" and "mamma," with a few oaths. Could make his wants known to family. Earned living by work on farm till fifty. Grew more childish and irritable later in life. Confused spells with hands raised to head at various times throughout life. Epileptic convulsions the last seven years of life. Occasionally noisy and homicidal. On admission, slight swaying in Romberg position. Knee-jerks dull, but equal. Other deep reflexes diminished. Superficial reflexes not obtained. Pupils small, oval perpendicularly, reacted within narrow limits. Petit mal and occasional severe epileptic convulsions during hospital stay (eight months). Death nine days after taking to bed with increased stupidity and signs of pneumonia in lower back.*

The autopsy † showed death due to typhoid fever and lobar pneumonia. The trunk showed little else abnormal, save half-sized testes, one containing a small cyst. There was coronary arteriosclerosis of moderate grade. The skull was symmetrical, the diploë congested. The pia mater was everywhere clear. The cerebrum showed anomalous fissuration, symmetrical in the anterior portions, both lateral and mesial, of both hemispheres. The affected lobes showed not only a microgyria with obscuring of normal markings, but also multiple fine corrugations of each gyrus with the thin pia mater exactly lining each irregularity.



The cerebellum showed an absence of lamination in a

* Clinical notes through kindness of Dr. C. B. Sullivan, assistant physician to the Danvers Insane Hospital.

† Autopsy notes and cerebellum through kindness of Prof. A. M. Barrett of Ann Arbor, Mich., during whose service as pathologist to the Danvers Insane Hospital the case came to autopsy.

broad area over the upper surface (see text-figure). The parts without laminæ were the following: Anterior half of culmen monticuli, inner two-thirds of left lobus culminis, inner two-thirds of left lobus clivi, a narrow strip in the left lobus cacuminis posterior to the postclival fissure, inner third of right lobus culminus, inner third of right lobus clivi (with preservation of laminæ for a narrow strip anterior to postclival fissure). The clivus and the substance adjacent to the posterior cerebellar notch showed normal lamination.

The microscopic examination of the non-laminated region of the cerebellum in this case showed interesting translocations of various structures. Isolated cells and groups of cells of the Purkinje cell type exhibit how little necessary relation exists between the development of Purkinje cells and that of the granule layer. Purkinje cells occasionally abut upon stray fascicles of white matter, as demonstrated in Weigert myelin sheath preparations.

The cell groups representing the granule layer are, as a rule, rounded like the normal granule layer, but occur in rather slender, anastomotic or isolated masses, as a rule, bordered by Purkinje cells (see Plate V., Fig. 4). There are occasional complete rings of cells, grouped to imitate the normal granule cells with cells of the Purkinje cell type both inside and outside the ring (see Plate V., Fig. 5). The cell-richness of these abnormal granule-cell accumulations is within normal limits. The acidophile structures of Denis-senko are occasionally seen.

The molecular layer seems to be more irregular in development than the other layers and is subject to more sudden changes of contour. A layer roughly resembling the molecular layer invests the flat surface of the region affected and in some cases abuts directly upon fan-shaped masses of medullated fibers.

The case brings out the possible independence of development of the granule layer and the Purkinje cell belt (see Plate V., Fig. 6). In a case of severe early developmental injury, the general cell relations (possibly the general effectiveness of the apparatus) can be altered, yet the granule layer

preserves independence as if it were to some degree a tissue unit.

CASE III. C. K., salesman of forty-seven years, entered Dr. G. B. Shattuck's medical service at the Boston City Hospital Dec. 22, 1902. Alcoholic, obese. At thirty-seven in bed four to five weeks with rheumatic fever, at forty-one in bed four weeks with scarlet fever, at forty-three in bed eight weeks with broken leg.

Patient took to bed five days before entrance with weakness and anorexia.

On entrance apathetic, râles in lower right back, abdomen tympanitic, distended, Babinski reflex on both sides, leucocytes 20,000. Delirium next day, at first restless, later more quiet. Rose spots December 26th. December 28th difficult to rouse, quiet, numerous rose spots, fever, respiration rapid and difficult, leucocytes 8,800. Widal reaction absent. The râles increased, respiration became difficult, mucus could not be coughed up, cyanosis developed. Death eighth day after entrance, thirteenth day after symptoms developed.

The autopsy showed: Brain wt. 1,190 grams. Dura quite adherent to calvarium. Numerous pacchionian granulations along longitudinal sinus. Pia injected. Large amount of subpial fluid. Cortex soft and edematous. White matter normal. Lateral ventricles contain more fluid than normal. Lower portion of right hemisphere of cerebellum contains six or eight minute hemorrhages, two of them extending into dentate nucleus.

DIAGNOSES. — Typhoid fever; acute splenitis; lymphnoditis (ileo-cecal group); acute bronchitis; early pneumonia; edema of cortex; suppurative otitis media; chronic interstitial orchitis.

The microscopic examination of the cerebellum in this case showed small hemorrhages into the molecular layer derived from small pial vessels, as a rule at the base of sulci. The molecular layer itself showed little reaction beyond slightly more vesicular nuclei in the neuroglia cells and pyknotic nerve-cell nuclei.

The reaction in the interior layers beyond the zone of destruction is striking (see Plate VI., Figs. 7 and 8). For here, whether through pressure or poison, there is evidence of differential cell-destruction. Whereas the lamina as a whole is quite normal in cell supply, the region opposite the effusion of blood shows death of Purkinje cells and a sharply marked zone of granule cells with pyknotic nuclei. The acidophile structures of Denissenko are, however, not visibly altered.

The case is in effect a natural experiment to show that

differential destruction of cellular elements occurs in the cerebellum and to give an idea of the order of cell destruction.

CASE IV. A poorly developed and ill-nourished male of twenty-five years. Never a bright scholar, mill worker. Albuminuria and defective memory three years before commitment, six years before death. Exaggerated knee-jerks, slow hesitant movements, complete disorientation, imperfect insight into condition, diminished effect. Hallucinations elicited two years after commitment. Death with unchanged signs.*

The microscopic examination of the cerebellum in this case showed the granule layer in numerous laminæ infiltrated with deposits of hyaline and lime salts (see Plate VII., Fig. 9). The granule cells are locally thinned out. The granule cells which remain are well preserved. There is a considerable supply of fibrillar neuroglia tissue throughout the laminæ both near the lime deposits and remote from any. Similar small lime deposits lie in the white matter near the granule layer. The acidophile structures of Denissenko are preserved.

CASE V. A. A., theater-employee of thirty-two, entered Dr. J. W. Bartol's service at the Boston City Hospital Aug. 25, 1905. The patient had been subject to diphtheria and scarlet fever in youth, besides "bilious" attacks followed by jaundice and vomiting. Gonorrhea. Syphilis denied. At twenty-eight the patient had an attack of headache, vertigo, nausea, and vomiting, lasting at least a fortnight. Two attacks afterward. All three were regarded as due to nephritis.

Four weeks before entrance (five weeks before death) occipital headache, vertigo, anorexia, pain in back, palpitation and dyspnea on exertion, cramps in feet at night, and increased amount of urine appeared. Gave up work eight days before entrance. Vomiting all one day four days before entrance.

On entrance, uremic odor to breath, systolic murmur in mitral region, marked arteriosclerosis, knee-jerks present and equal, spurious ankle-clonus. Convulsion lasting fifteen minutes the day following entrance. Swelling of eyelids. Twitching of head and arms. A swelling size of fist on right side of neck developed September 2. The patient became worse in the evening and died an hour later having developed considerable rigidity of neck, Kernig's sign, increase of knee-jerks, bilateral ankle-clonus.

The examination of the head † showed normal scalp, calvarium, and

* Clinical notes through the kindness of Dr. H. W. Mitchell, senior assistant physician to the Danvers Insane Hospital.

† I am obliged to Dr. C. W. Duval, pathologist to the Montreal General Hospital, for the tissues of this case, examined by him while assistant in pathology in the Boston City Hospital.

dura, well marked arachnoidal villi and basal vessels without gross sclerosis. Considerable subpial edema. Brain weight 1,435 grams. Substance uncommonly firm. Small cysts of choroid plexus. Pons, medulla, mid-brain, basal ganglia negative on section. Cerebellum showed nothing abnormal on section.

The aorta showed sclerosis without calcification. There were small old foci of calcification in the liver, and small calcareous masses in the pyramids of the kidneys. Chronic diffuse nephritis. Chronic splenitis. Death was due to acute purulent pericarditis.

The microscopic examination of the cerebellum in this case showed lesions similar to those of Case IV., but of severer character (see Plate VIII., Figs. 10 and 11). The acidophile structures remain intact. Numerous active fibril-producing astrocytes are found in the granule layer. The fibrils often embrace the acidophile structures closely, but apparently fail to pierce them (see Plate VIII., Fig. 11).

CASE VI. Undersized negro, of twenty-three years. Hutchinsonian teeth. Backward at school, of limited mental capacity. Tendency to fall down at play. Change for the worse asserted during last five or six years of life. Sold newspapers or blacked shoes up to within a year of death. In last months of life, showed general incoördination (coarse drunken movements) and tremors, with shifting gaze and tendency to nystagmus. Disorientation for time, place, and persons, confusion with coarsely relevant replies to questions, ill-defined and transitory delusions of persecution, auditory, visual, and tactile hallucinations, defective memory. A few remissions with slight clearing up of confusion.

The autopsy showed death due to an early bronchopneumonia (numerous staphylococci). The organs of the trunk were small and firm (heart, 130 grams; spleen, 25 grams; liver, 640 grams; kidneys together, 150 grams), and suggested, as did the brain, a hypoplasia. There was persistent thymus tissue in a tongued shaped mass, measuring 3×2×0.5 centimeters above the aortic arch. The femoral marrow was red and jelly-like, suggesting red bar-le-duc. The arch of the aorta showed a few plaques of sclerosis. The brain weighed 965 grams and showed chronic diffuse and focal leptomeningitis roughly corresponding with a diffuse cerebral and cerebellar gliosis. The cerebrum also showed focal sclerotic nodules, resembling those described in idiots by Bourneville, in the temporal gyri. Occipital microgyria. Gliotic lesions with pigmentation in posterior horns of spinal cord, perhaps related with a hydromyelia demonstrable at some levels.

The cerebellum was as firm as the normal olivary bodies. The hemispheres were symmetrical and of a normal appearance, save that the

laminæ were slightly narrower than usual and very compactly set. The pia mater showed a diffuse haziness, best marked over the superior surface, besides focal thickenings of an unusual character, linear mounds of fibrous tissue running at an angle to the laminæ. There was no notable overgrowth of fibrous tissue at the base.

The microscopical examination showed in the cerebellar cortex the greatest possible gliosis consistent with maintenance of normal topography.

The meninges show an infiltration of moderate degree with cells of the lymphocyte series and pigment-bearing cells, together with edema over the most contracted lamellæ.

The vessels of the central white matter near the origins of the laminæ often show collections of granular material (possibly lime salts) ensheathing the intima. This process is less common in the meninges.

Some of the lamellæ have preserved a good number of Purkinje cells. Numerous marginal lamellæ show no Purkinje cells. Occasional Purkinje cells show two or more nuclei.

The molecular layer shows everywhere a notable increase in Bergmann's fibers, and in many lamellæ is diminished one-third or more in depth. A number of marginal lamellæ show a deep superficial zone of concentric fibrils in the locus of the fetal cell layer; this new layer is unevenly developed and recalls the (somewhat more richly cellular) focal superficial gliosis of the temporal cortex cerebri of the same case.

The granule layer shows a diffuse aplasia, and a thinning out of the granule cells, especially well shown toward the extremities of the lamellæ. The granule cells preserve in many places their usual grouping, but the picture gradually changes to one of granule cells of focal occurrence only or to one of complete loss of granule cells. The degree of fibrillary gliosis seems to parallel the loss of granule cells.

The white center shows a less dense gliosis (replacement gliosis) not in particular relation to vessels with occasional cystic deficiency near tips of laminæ.

Cerebellar cortex (myelin sheath stain). — In some cases almost total absence of fibers in centrum album gyrorum,

in many cases pronounced thinning out toward the tip. A few fibers are almost everywhere preserved in the molecular layer. (Marchi) — Black granules, larger and less even in distribution in the centrum album than in the cerebral cortex by the same method.

Dentate nucleus. — High neuroglia fibril content about large nerve cells and notably within the nucleus (see especially phosphotungstic acid hematein preparations). The Marchi preparation shows large scattered drops particularly within nuclear limits.

I have been able to collect a few notes from the literature concerning the granule layer of the cerebellum in disease.

Bergmann,¹ 1861, described findings in an atrophic cerebellum, which, as Weigert has pointed out, are in part erroneous, since Bergmann regarded the radial fibers which now bear his name as forming a reticulum. Bergmann also described a paling-out of the granule layer and a diminution in number of granule cells.

Obersteiner,² 1871, described a focal lesion in the right cerebellar hemisphere in a man of thirty years dying of acute yellow atrophy of the liver. Obersteiner there called attention to the absence of Purkinje cells, to the development of numerous radial fibers in the molecular layer, and to a layer of concentric fibers (which he terms Ependymschicht) in the inner portion of the molecular layer. The Purkinje cell layer is figured as full of gaps and without cell accumulation. Obersteiner compares the granule layer to lymphoid tissue, remarking that the granule cells are held in a sponge-like ground substance. He found, also, in the granule layer a few larger elements which he regarded as degenerate nerve elements (one possessing two nuclei).

Kirchhoff,³ 1882, described two cases of atrophy and sclerosis of the cerebellum, in a girl of seven (symptoms beginning at five years) and in an imbecile woman of twenty. Kirchhoff compared the appearance of the atrophic area in the superior vermis and left cerebellar hemisphere in the girl to the appearance of the embryonic cerebellum at the sixth month. Kirchhoff compared the condition of the more extensive cerebellar atrophy in the imbecile with the appearance of the embryonic cerebellum at the seventh month. In both cases Purkinje cells were absent from the atrophic areas. From the even diameters of the granule layer throughout the sclerotic laminae in the imbecile, Kirchhoff argues the embryonic date and origin of the lesion. It is doubtful whether this argument can be upheld.

Sommer,⁴ 1884, described cerebellar atrophy and sclerosis in a hydrocephalic of thirty years. He found the granule layer reduced from 300 to about 60 in depth. The granule cells seemed normal but reduced in number and wide apart. In the area normally occupied by granule cells,

Sommer found a granular ground substance in which are irregularly scattered many rounded nuclei. The small vessels had thickened walls. Sommer regarded these changes and those in the white matter as secondary to the absence of Purkinje cells and other alterations in the gray substance.

A. Meyer⁵ (Osnabrück), 1890, figured diminution of nerve fibers in the cerebellar cortex, chiefly in cases of general paralysis, by means of Weigert's myelin sheath method. With respect to the other elements Meyer says that carmine preparations demonstrated no other alterations in the outer or in the granule layers, and believes that there is no alteration or numerical reduction in the Purkinje cells. He regarded the degeneration as primary and analogous to similar alterations in the cerebral cortex.

Weigert,⁶ 1895, found "*so gut wie gar keine*" neuroglia fibers in the granule layer under normal conditions, and this in contrast with the Bergmann fibers of the molecular layer, the fibers surrounding the Purkinje cells, and the normally rich supply of fibers in the white matter. Weigert, however, mentions the increase of fibers in the granule layer in general paralysis and other conditions.

Raecke,⁷ 1901, described an examination of the cerebellum in fifteen subjects of general paralysis by Weigert's neuroglia method. Raecke confirmed Weigert's observation of an increase of Bergmann's fibers in the molecular layer. Raecke found a peripheral zone of gliosis and a gliosis about the Purkinje cells. It is curious that the Purkinje cells, according to Raecke, show little or no change except in the severest foci, and Raecke is apparently of the opinion that the Purkinje cell alterations are secondary to the gliosis. The process in the granule layer was studied in the severest foci. Here the lost granule cells are simply replaced with neuroglia. Raecke goes on to say that — "since with every overgrowth of supportive tissue, whether primary or secondary, there must correspond a certain loss of nerve elements," it is probable that the earliest change in general paralysis affects the dendrites of the Purkinje cells.

INTERPRETATION OF FINDINGS.—The cerebellum is a good field for the study of differential lesions. There is a striking variety of structures in it. Yet this structural variety is, roughly speaking, the same throughout. The obvious structural unit is the cerebellar lamina. Each lamina presents a white core containing ingoing and outgoing fibers. Surrounding the white core is the gray matter of the cerebellar cortex. The elements of the cerebellar cortex comprise, (1) an important centrifugal apparatus — the Purkinje cells; and (2) a number of structures regarded as centripetal — the various structures of the granule layer, together with sundry

elements of the molecular layer. The granule layer lies next to the white core and contains elements concerned presumably with the reception of impulses entering the lamina by way of the white core. The granule layer apparatus comes into relation with the manifold processes of the Purkinje cells which project into the molecular layer, in a fashion which is not obvious. The data of the Golgi impregnations here step in to demonstrate processes from the granule cells running outward to fall into relation with the dendrite forest of the molecular layer.

The course of a single impulse (an artificial abstraction in the cerebellum probably) might then be inward in a fiber of the white core to a granule cell, thence in a process to some dendrite of a Purkinje cell lying in the molecular layer, thence doubling on itself and running outward through the Purkinje cell and its single axis-cylinder to the white core again. It is of course doubtful whether any single impulse ever runs this uncomplicated course in the cerebellum. Not only have certain interposed elements been omitted in the series mentioned, but also it is highly probable that no single impulse goes far into the cerebellar cortex without starting up fresh impulses in several directions. The granule cell groups are precisely fitted for this, since little or no non-nervous material of the neuroglia type is discernible between the granule cells. Elsewhere in the cerebellum there are much more pronounced intervals between the nervous elements as well as a considerable supply of neuroglia which provides more effective surfaces of separation than the closely compact granule layer possesses.

Once running along Purkinje cell dendrites, it is difficult to see how a nerve impulse could be interrupted in its centrifugal course, although its intensity might conceivably depend upon the number of dendrites activated. If this conception be accurate, most of the transferent and inhibitory functions must be attributed to apparatus previously mentioned, namely, to apparatus centering in the granule layer.

Next to the granule cells the most prominent elements of the granule layer are the apparently homogeneous

eosin staining structures of Denissenko. These are buffer elements imbedded in the anastomotic granule cell masses. Their functions may be to heighten, to depress, or otherwise to modify impulses which reach the granule layer. They may or may not alter in condition from moment to moment. It is possible, if improbable, that these eosin structures have no nervous function.

I have been studying the cerebellum, not so much in the hope of directly unfolding its functions, as to show what are the relative resistances of its elements to disease. In a previous paper I have described once more the familiar fact that the great elements of the Purkinje cell belt are the first to undergo destruction. I have also pointed out that in many types of lesion the Purkinje cell belt is the initial scene of activity, which activity takes the form of neuroglia cell overgrowth. When a lesion proceeds no farther than destruction of Purkinje cells, it is evident that the consequent alteration of function must be relatively coarse, tantamount perhaps to a mere suspension of functional discharge from the injured focus.

I have therefore looked over a number of cases to find examples of more exquisite injury to the centripetal or central apparatus, the apparatus which must have much to do with the coördination and perhaps with the inhibition of impulses. I have here presented a few cases to illustrate points of this nature. The most interesting result of this study is the observation that the eosin-staining structures of Denissenko are remarkably stable structures in comparison with the other elements of the granule layer.

The discovery of nucleated structures in place of the non-nucleated structures of Denissenko in a case of maldevelopment (Case I.) seems to offer hope that the development of these structures may be worked out. It is possible that the eosin structures of Denissenko stand in the same relation to nucleated structures in early life in which red blood globules stand to blasts. Work on this line is in progress.

The observation that Purkinje cells can develop independently of granule cells, and vice versa (Case II.), seems to be

consistent with the conception of their functional independence outlined above. Granule cells appear to develop together and are supplied with the eosin structures of Denis-senko.

A case of typhoid fever with small hemorrhages in the cerebellar pia mater (Case III.) offered a natural experiment in which both the maximum lability of the Purkinje cells and the initial neuroglia activity of the Purkinje cell belt are again demonstrated. The case likewise presented in the same region of focal atrophy necrosis of granule cells, among which however the eosin-staining structures of Denissenko persist.

Two further cases (Cases IV. and V.) demonstrated a curious deposition of hyaline material and lime salts confined largely to the granule layer. The lesion, of interest in itself, is here used to demonstrate the point that the eosin-staining structures persist despite the atrophy and death of numerous granule cells. In one case a delicate neuroglia overgrowth occurred, and the neuroglia fibrils are found embracing but not piercing the eosin-staining structures.

Work is in progress in the direction suggested by Case IV. Such a case demonstrates the possibility that, despite normal afferent nerves and a normal discharging apparatus (the Purkinje cells), there may be disorder of the most exquisite character in the central receptive apparatus (granule layer) of the cerebellum.

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DESCRIPTION OF PLATES.

PLATE IV.

FIGS. 1, 2, 3. — Cerebellum in Case I. (case of cystic aplasia of the cerebral hemispheres with absence of pyramidal tracts).

FIG. 1. — Note the relative cell-richness of the granule layer. Compare with the normal cell supply of the granule layer as shown in Fig. 8.

FIG. 2. — Same case as Fig. 1. Granule layer showing supernormal cell-richness as compared with the adult. Note spaces between the granule cell clusters. Spaces contain single central nuclei.

FIG. 3. — Same case as Figs. 1 and 2. Large cells of white matter, one containing two nuclei and dark-staining central bodies. These cells are probably of the neuroglia series and possibly of the same type as those filling the clear spaces of the granule layer shown in Fig. 1 and particularly in Fig. 2.

PLATE V.

FIGS. 4, 5, 6. — Cerebellum in Case II. (Case of cerebellar anomaly: absence of lamination in part of superior surface of cerebellum in an epileptic imbecile).

FIG. 4. — Note irregular contours of tissue representing the granule layer. Heterotopia of Purkinje cells.

FIG. 5. — Heterotopia. Granule layer ring with Purkinje cells both inside and outside.

FIG. 6. — Group of Purkinje cells growing independently of the granule layer.

PLATE VI.

FIGS. 7, 8. — Cerebellum in Case III. (case of typhoid fever with small hemorrhages in the pia mater of the cerebellum). The two photographs are from the same lamina. Fig. 7 is taken from the injured side of the lamina, Fig. 8 from the uninjured side. The differences are due to the presence of a focus of pial hemorrhage opposite Fig. 7, absent opposite Fig. 8. The hemorrhage is not shown in Fig. 8, which exhibits its effects across the intervening molecular layer. Fig. 8 shows

1. Persistence of but one Purkinje cell, which was intensely eosinophilic and shows an eccentric nucleus;
2. Sparse and pyknotic granule cell nuclei in one focus adjacent to the Purkinje cell belt; and
3. Numerical increase of neuroglia cells in the Purkinje cell belt itself.

The lesions of Fig. 7 are shown nowhere else in the affected lamina or in adjacent laminæ. Fig. 8 shows essentially normal appearances.

PLATE VII.

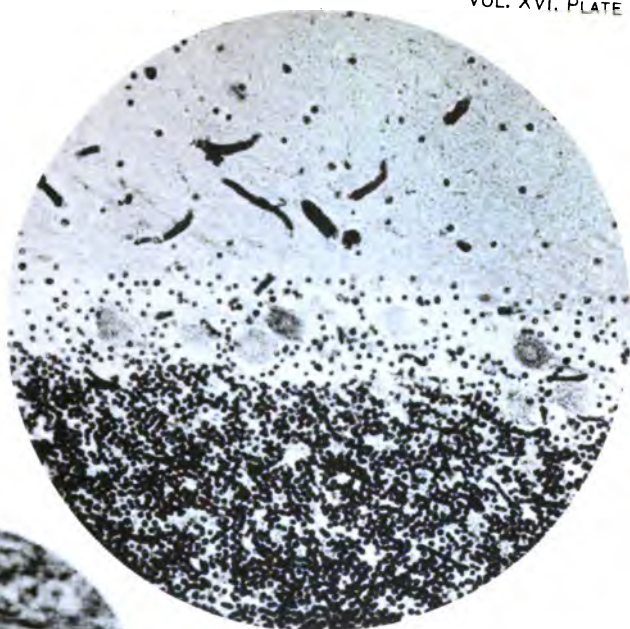
FIG. 9. — Cerebellum in Case IV. (case regarded as dementia præcox). Lamina with coarse masses of lime, locally replacing groups of elements in the granule layer. Besides the large masses numerous smaller masses occur throughout the granule layer, and there is a diffuse delicate fibrillar gliosis throughout.

PLATE VIII.

FIGS. 10, 11. — Cerebellum in Case V. (case without nervous symptoms dying of acute purulent pericarditis. Foci of calcification found also in liver and kidneys). Compare Fig. 9. Marked atrophy of elements of granule layer. The granule cells preserve their arrangement in clusters. Small homogeneous apparently structureless bodies, slightly larger than granule cells, of a rounded or oval shape in section — structures (so-called "cells") of Denissenko — persist. Fig. 11 shows a swollen fibril-producing neuroglia cell. The fibrils stretch out to embrace a persistent structure of Denissenko.

PLATE IX.

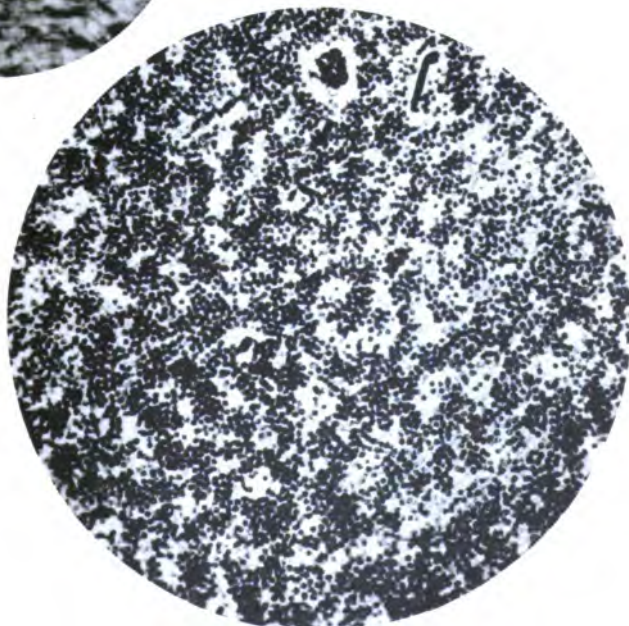
FIGS. 12, 13. — Atrophy of granule cells with volume of layer largely maintained. Structures of Denissenko persist.



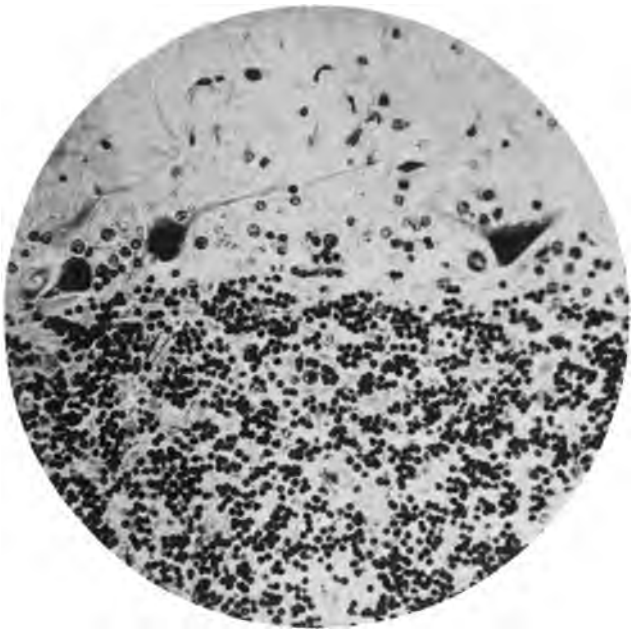
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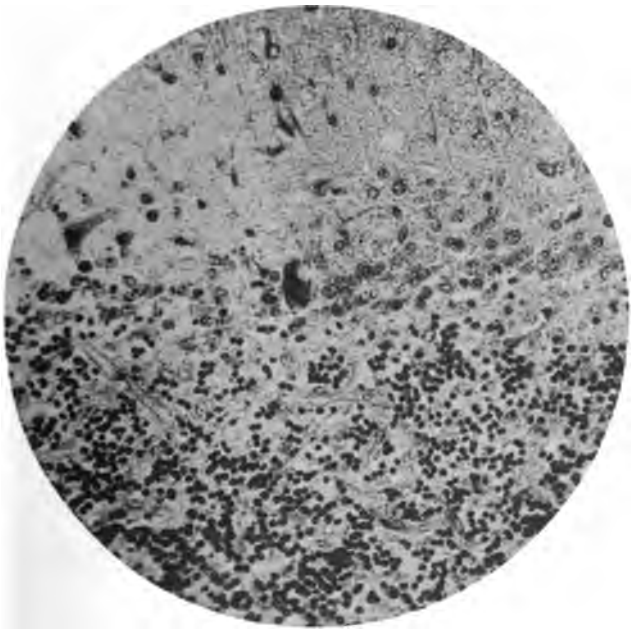
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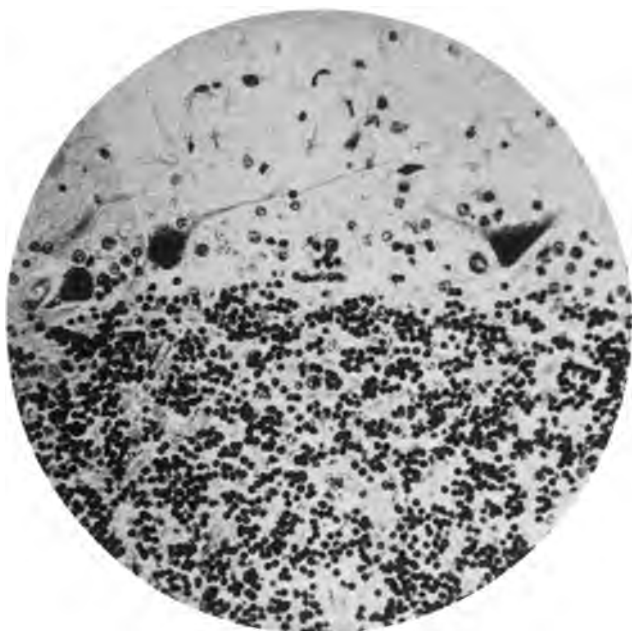


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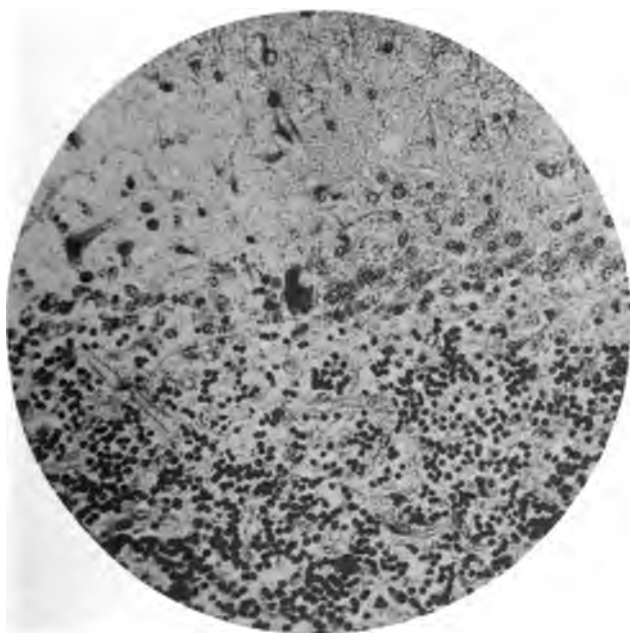


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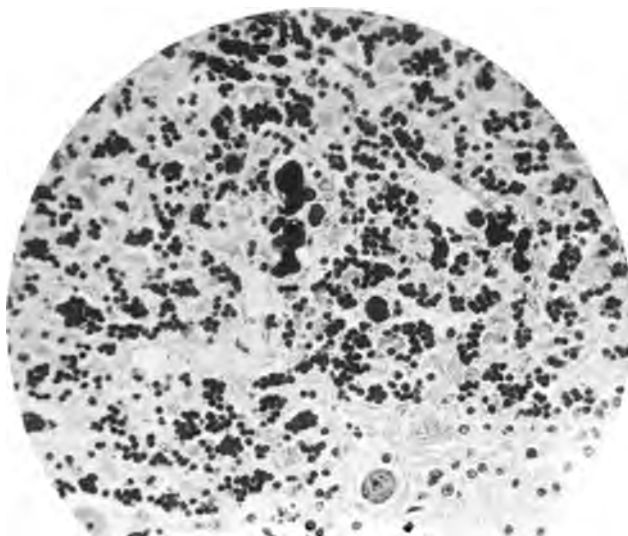
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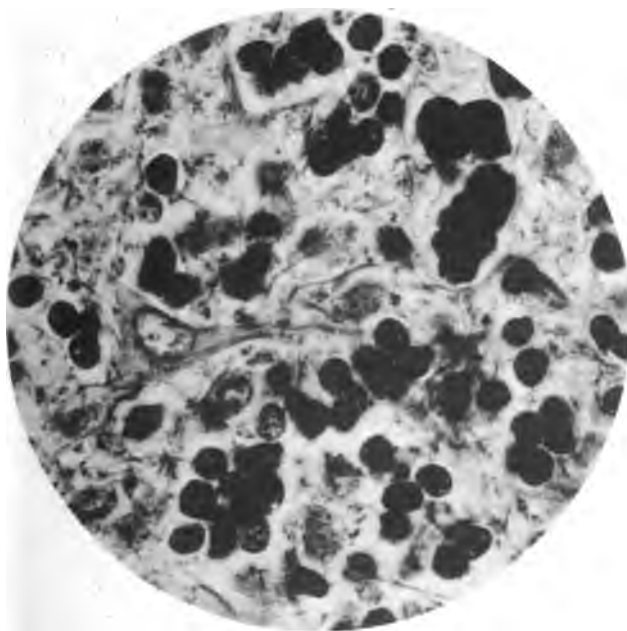
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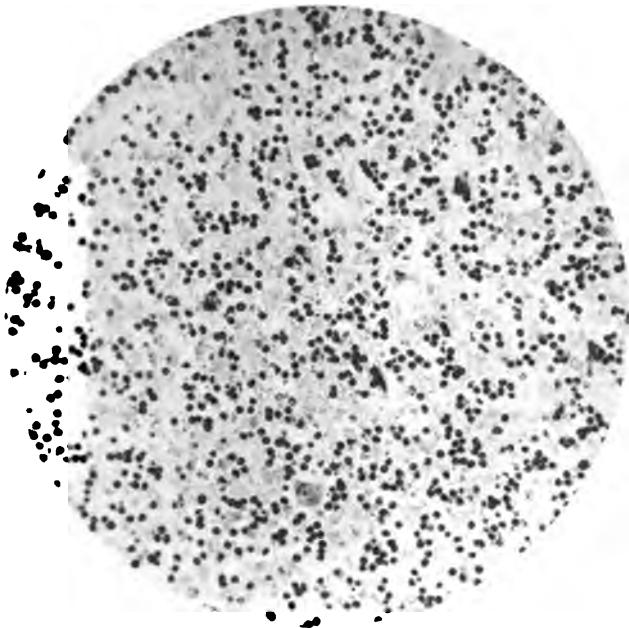
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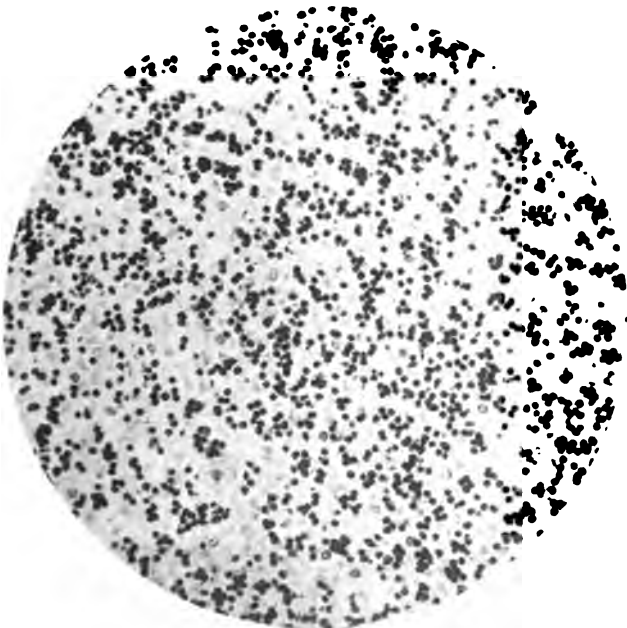
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13

ON THE OCCURRENCE OF OXIDATIVE FERMENTS IN A
MELANOTIC TUMOR OF THE LIVER.*

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Enzymes capable of oxidizing aromatic hydroxyl compounds to black substances of the melanin class occur very frequently in plants. In animals such enzymes have been found less frequently. They are known to exist in the blood of insects and crustacea and to their action the darkening of the blood of these animals on exposure to air has been attributed. In the lining of the ink-bag of the squid a similar enzyme has been found. Most of these enzymes have the power of oxidizing tyrosin to a black substance, and are therefore known as tyrosinases. In some instances the enzyme is unable to attack tyrosin, though able to attack the more easily oxidizable aromatic hydroxyl compounds, as for instance hydrochinon. Of this nature was the enzyme discovered by Pieri and Portier¹ in the gills and antennæ of acephalæ. In vertebrates no such melanin-forming enzyme has hitherto been described. v. Fürth² suggests that enzymes of this class occur perhaps in all animals. He suggests further that the most promising place in the higher animals to search for them is in melanotic tumors. Here much pigment is formed in a short time, for the amount excreted daily in the urine in such cases may be considerable.

When therefore through the kindness of Professor Mallory I came into the possession of a much enlarged human liver which was colored uniformly and diffusely black, I endeavored to discover in it an enzyme capable of oxidizing aromatic hydroxyl compounds. As Dr. G. B. Magrath who performed the autopsy kindly informed me, the primary tumor was probably situated in the eye, for the patient had shown early ocular symptoms. The opening of the head was not allowed. The patient had also passed urine containing melanin.

* Received for publication Jan. 25, 1907.

The liver was finely ground up and the juice squeezed out in a press. The juice was quite black and opaque. Many attempts were made to remove the pigment without injuring the enzymes, but they all failed. The attempts to get a pale extract from the liver pulp remaining behind in the press were also unsuccessful. The aqueous solutions as well as the glycerin extracts were quite black. An attempt to remove the pigment by means of fractional precipitation with ammonium sulphate was unsuccessful because at concentrations, which had removed nearly all the coagulable proteid, the filtrate was still colored. The greatest amount of pigment was removed by acidifying, though the filtrates were still very dark and the acid seemed to exert a deleterious influence upon the enzymes. I was therefore compelled to use a very little of the expressed juice or the extract in a considerable quantity of the solution of the aromatic compound. Even then the mixture had a light dirty gray color.

The method of procedure was as follows: To about twenty cubic centimeters of a saturated aqueous tyrosin solution, or a fairly strong aqueous pyrocatechin solution, a few drops of the expressed juice or the extract were added. The mixture was then divided into two parts, of which one was boiled. Both were placed in the thermostat over night at 37° C. In the morning the unboiled pyrocatechin was invariably very much darker than the boiled control. In some cases it was quite black and opaque. In the bottom of the test-tube there was usually a gray-black precipitate which appeared to be a mixture or compound of the proteid contained in the extract with the oxidized pyrocatechin. In the case of tyrosin the results were less clean cut. Sometimes no appreciable darkening of the unboiled solution could be observed. In other cases there was a distinct though slight darkening. Whether the enzyme acts upon tyrosin must therefore be left undetermined, though it seems probable that the inconstancy of the results was due to the great dilution in which the enzyme had to be used. As a control the action of the expressed juice of a normal human liver and its extract were tested upon both tyrosin and pyrocatechin

in exactly the same way. In all cases the results were negative.

Though it is clearly realized that it is unsafe to generalize from a single case, nevertheless it was thought wiser to record these results as a stimulus to further work along these lines than to wait years in order to accumulate more of these rather rare cases.

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FATTY TUMOR OF KIDNEY SUGGESTING A METAMORPHOSIS
OF ADRENAL CELLS INTO TRUE FAT.*

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While heterogeneous tumors by their very obscurity, as well as by their suggestiveness from the histogenetic standpoint, must always be interesting, it would seem that a somewhat special interest might be attached to the finding of fatty tumors in the kidney.

To find in the kidney, in which normally fat is absent, a tumor consisting wholly or largely of fat naturally awakens conjecture. Possibly it was this direction of thought that led Grawitz¹ to investigate more accurately those fat-like growths of the kidney which Virchow had described as pure lipomata. Since his important discovery in 1883, that these apparent lipomata were in many cases misplaced and overgrown adrenal structure, a large number of articles have appeared, for the most part confirming, enlarging, and modifying his work.

Now while the great majority of these tumors (since described), arising in adrenal rests, are typical and easy of diagnosis upon a reasonably careful microscopical examination, there have been a few which by virtue of containing a certain amount of typical fat, and not merely adrenal cells filled with fat, have occasioned doubt as to whether they were lipomata or hypernephromata; there have been other growths described as undoubted pure lipomata, others again as fibro-lipomata, angio-lipomata, or lipo-myomata, all however containing fat as their principal element. In order to clear the ground for our own case, it may be well at this point to review briefly this aspect of the subject.

* Received for publication Jan. 28, 1907.

In analyzing the literature of fatty tumors of the kidney we have been able to distinguish several classes of growth, some well established, others depending on a theory of the author concerned. First we have the typical Struma suparenalis aberrans of Grawitz, since known under the more convenient name of Hypernephroma (Birch-Hirschfeld). This is a tumor which is fatty only upon macroscopical observation. Dissolve the fat with alcohol during preparation for stained sections and one finds macroscopically large cells very deficient in protoplasm (the latter having been replaced by fat) well named by Albarran "the large clear cells," which are frequently arranged in cylinders or columns so as to resemble markedly the Zona fasciculata of the adrenal cortex, and are very apt to contain glycogen. In short, a picture which to pathologists nowadays is so well known as to render further description on our part unnecessary. Neither is it our intention to describe here the variations of growth which hypernephromata may exhibit; that is, the sarcomatous or the carcinomatous variation with the disappearance of fat, the hemorrhagic and cystic degeneration, the malignant type of metastases, and so on. What we wish here to keep prominent as the type of this class is the ordinary hypernephroma which, by virtue of its fat content, used to be and macroscopically may still be mistaken for true lipoma.

In the second place, there is the true lipoma. After Grawitz's discovery the tendency was to revise former diagnoses and set down all previously described lipomata as hypernephromata. Soon, however, a proper investigation showed that true lipomata did occur in the kidney, but that they were rare and never exceeded the size of a cherry. Selter,² who recently reviewed the older cases critically, came to the conclusion that of these the larger ones were all instances of replacement lipomatosis, arising from the fat round the pelvis of the kidney, as the result of irritation from calculus; at least he found a calculus present in all but two of the cases, while the smaller ones were practically herniated from the capsule into the kidney tissue. Grawitz

and Horn³ had previously come to the same conclusion as to the larger tumors — in Grawitz's words, that the fat was "perinephritic and homoöplastic."

Virchow's theory concerning the development of fat is well known. Writing in his "Onkologie" upon lipoma of the kidney (and as a matter of fact, one at least of his cases was a true lipoma according to Grawitz's later investigations), he said: "In this organ fat is heteroplastic. When one can follow its development clearly it arises in the same way as fat tissues generally; that is, there occurs first a proliferation of connective tissue, then the new-formed cell agglomerations change into a fat lobule by taking up fat in the interior of the cells. In this way there arise fat nodules in the kidney up to the size of a cherry, consisting of fully developed, moderately vascular, and sometimes lobulated fat."

Beneke,⁴ Alsberg,⁵ and Warthin,⁶ among others, have expressed strongly their adherence to this theory, upon the basis of cases described by them. Beneke holds that, in sharp contrast with fatty infiltrated adrenal rests, there do occur in the kidney true lipomata, which grow by a gradual "lipomatous infection" of the renal connective tissue, with simultaneous necrosis of the parenchyma and glomeruli. "Macroscopically," he says, "the small, soft, yellowish tumors found in the kidney may turn out microscopically to be either true lipoma, true adenoma, or adrenal rest, with or without adenomatous overgrowth."

Of these tumors described as lipomata apparently only four attained any material size: Alsberg's,⁵ Grawitz's,⁷ Warthin's,⁶ and Bartsch's.⁸ Warthin's case was a most remarkable one. The tumor was fourteen inches long, eight inches wide, and six inches thick; it weighed two pounds. It was essentially a fibro-lipoma which had begun probably in the connective tissue surrounding the large blood vessels in the boundary between cortex and medulla, and had grown chiefly towards the pelvis away from the cortex. It possessed no well marked capsule, but invaded the kidney in such a way as to cause atrophy of the latter while filling up the pelvis and projecting some distance down the ureter.

Microscopically it showed all variations between hard fibromatous and pure fat tissue, the one element predominating over the other according to the area in which the section was taken. Nowhere could be found any evidence of adrenal cells, or tissue of any kind. The blood vessels were rather numerous and large, with thick walls, but had not undergone hyaline nor amyloid degeneration. There were no areas of hemorrhage nor deposit of pigment.

Grawitz's case, which he entitled "Angio-myo-lipoma of the kidney" was a tumor of the size of a man's head. It replaced most of the right kidney leaving the poles free. There had been no blood in the urine. It measured $23 \times 19 \times 12$ centimeters and weighed twenty-two hundred and fifty grams; it extended from the hilus to the cortex and was situated inside the kidney capsule. Its main constituent was fat, and Grawitz believed that it probably arose from the fat of the pelvic region. It was not an infiltrating growth, but there was a fibrous compression capsule around it of kidney tissue. Some areas of the growth gave the appearance of an arterial angioma, with such abundant growth of smooth muscle fibers that the picture reminded one of a myoma; but even in these areas fatty lobules were inserted, and the main mass was lipomatous. Grawitz called it a rarity of the first order. It was the first case of lipoma of the kidney that he had seen which demanded operation. From his description the tumor evidently belongs to the class denominated by Mueller, mixed fatty tumors of the kidney, inasmuch as it contained muscle, although mostly lipomatous.

In Bartsch's case (we quote from Albarran, not having access to the original) the tumor formed with the kidney a mass twenty-five centimeters long, nineteen centimeters wide, and twelve centimeters thick; its weight was twenty-two hundred and fifty grams. It contained some smooth muscle tissue, in spite of which Albarran accepts it as a pure lipoma. In our opinion it also comes more naturally into the class of growths denominated by Mueller mixed fatty tumors of the kidney.

The kidney, in Alsberg's case, was the size of a child's

head. Upon section the renal substance was found to be studded with tumor nodules of a yellowish color, which, in the fresh state, showed a fatty luster. They were scattered fairly evenly through the organ, both in the cortical and medullary areas; their size varied greatly, from that of a millet-seed up to that of a walnut, and they were mostly of a globular shape. The larger nodules were sharply delimited from the kidney substance and could be easily enucleated. The renal tissue was not much altered, save that near the nodules it was usually grayish, and in spots soft and friable. Microscopically, many of them consisted of pure fat; in others, besides the fat, abundant fibrous tissue, giving a structure resembling that of subcutaneous tissue. But most remarkable of all were, in Alsberg's opinion, certain areas, found near each nodule, of dense, young, fibroblastic tissue, resembling somewhat spindle-celled sarcoma, in which in places the vessels were greatly overgrown. In these "fibroblastic clumps" there were to be seen a few fat cells here and there, and Alsberg considered that the latter was formed from the former, according to Virchow's theory. All these nodules were practically identical, one with another, with one exception, which proved to be, microscopically, a typical adrenal rest.

This observation, of the simultaneous yet independent presence of pure lipoma and pure adrenal rest in one kidney, had been already made by Grawitz and by Horn. According to the former, the explanation lies in a "simultaneous inclusion." In one of Horn's cases, and also in one described by Mueller,⁹ the nodule was composed, microscopically, half of pure fat and half of typical adrenal tissue, the one *not* shading off into the other. It may be remembered in passing that of Horn's cases, Nos. 13 and 14 showed no slight resemblance to our case; a detailed comparison may be reserved for the later discussion. These "simultaneous inclusion" cases we are inclined to place in a group by themselves, the third group.

A fourth group of tumors, in which fat is the principal element, is found in the so-called "lipomatous mixed growths

(Mischgeschwülste) of the kidney," described particularly by Mueller.⁹ It must be remarked, parenthetically, that the "mischgeschwülst" of the kidney indicates usually among pathologists that very malignant form of growth which is often called adenosarcoma and belongs almost exclusively to infancy and childhood. From these Mueller's small lipomata or lipo-sarcomata described below must be clearly differentiated. The latter belong to adult life mainly and are easily explained as capsular inclusions, while the former may perhaps be better explained by Wilm's well-known theory of very early misplacement. In certain cases of kidney tumor, which were apparently either pure lipomata, or lipomata in combination with sarcomatous growths, Mueller found muscle cells — at least he interprets them as muscle cells — sometimes in very small numbers, sometimes forming a considerable part of the growth. This observation, it may be remarked, had already been made by Lubarsch¹⁰ and Manasse.¹¹ These muscle cells Mueller found most frequently in the fibrous tissue separating the tumor from the kidney substance, a circumstance which suggested to him that the tissue must be merely part of the kidney albuginea misplaced; and in this he found a support for his argument that the tumors in question were heterotopic, representing a dislocation of fragments both of the fatty and the fibrous capsule of the kidney.

In one case, already referred to — a horseshoe kidney — Mueller found a nodule of the size of a mark-piece, which contained side by side, yet independent of each other, fat and adrenal tissue. There were no transition pictures. The fat, however, seemed to be sending out processes into the adrenal structure, as if it were beginning to overgrow the other; from which Mueller concluded — on quite insufficient grounds — that ultimately a pure lipoma might have resulted; not, however, be it expressly remarked, as the result of the fatty degeneration of the adrenal epithelium, for in that he plainly states his disbelief, but rather through an active overgrowth of the fat and consequent destruction of the adrenal portion by it.

Horn's cases (Nos. 13 and 14) might also be described

at this point inasmuch as, although containing no muscle tissue, his explanation of the findings corresponds with Mueller's theory as given above. Number thirteen was a small yellowish nodule, 1.75 centimeters in diameter, lying in the lower pole of the left kidney. It was situated underneath the capsule and extended from there into the pelvis. It had no true capsule and its color varied from yellow in the center to white or red-brown at the periphery. The main mass consisted of spindle cells and vessels, but at the center there were large spaces about the size of a fat cell filled with large and small fat droplets, while at the periphery there were rows and double rows of epithelial cells identical in size with the fat filled spaces and even a few epithelial cells were to be seen here and there between the spindle cells.

Number fourteen was a small yellow tumor lying underneath the capsule and not separated from the kidney tissue. The greater portion of this tumor consisted of regular fat tissue, but there were also rows and double rows of polygonal epithelial cells undoubtedly of adrenal origin. He considers both these to be instances of contemporaneous development of two unrelated tissues, that is, true fat and adrenal tissue.

A fifth and last group is constituted by such collections of fat in the kidney as Ulrich¹² described. His observations were made upon five cases, in none of which was the fatty growth larger than a cherry. He contests Virchow's theory, which would have the fat to develop by metaplasia out of proliferated interstitial connective tissue; for this there is not enough proof, he says, though he admits its possibility theoretically. On the other hand, the Grawitz idea of dislocation or inclusion of portions of the fatty capsule, while possible, is unlikely. Excluding these theories he comes, by a careful microscopical examination of his five cases, to the conclusion that these so-called lipomata are really nothing but a very loose connective tissue surrounding localized areas of urinary tubules, of which the tube-like spaces are filled with fat, this fat being a sort of substitution product replacing destroyed kidney epithelium, and forming, by confluence, larger or smaller fat globules. In short, the so-called

lipomata, Ulrich insinuates, are in most cases, if not in all, mere degeneration products, if the term may be so used; in other words, a sort of replacement lipomatosis.

To summarize, we have then five classes of fatty growth in the kidney:

1. The typical hypernephroma, looking like a lipoma;
2. The true lipoma;
3. The combination of these two in one nodule, lipo-hypernephroma, if we may call them so, each element remaining separate;
4. The lipo-myoma, or lipo-myo-sarcoma of Mueller, and
5. The degeneration lipoma of Ulrich.

The tumors hitherto described under these various types have all been very small, not larger than a cherry, or at most a walnut, with the four exceptions already mentioned. But even in Alsberg's case, the largest of the individual new growths in the kidney was no larger than a walnut. Warthin's resembles our own in size. Those of Grawitz and Bartsch are really mixed tumors in the sense of containing muscle as well as fat, but are included in the present summary because of their having attained a much larger size than any others reported, and because of their being chiefly lipomatous in nature.

Having now indicated briefly the nature of the various fatty growths of the kidney hitherto described, and the theories propounded in explanation of their genesis, we may proceed to describe the tumor that forms the basis of this article, and, later, attempt to establish the differential diagnosis.

The patient, Mrs. A. D., from whom the present tumor was removed, entered the Royal Victoria Hospital in February, 1905, complaining of a lump in the right side of the abdomen and of a dull dragging pain felt in the same region. She had noticed the mass accidentally about May, 1903, since then no change in size, as far as patient could judge, took place. About August, 1903, patient commenced to have occasional slight attacks of dull pain, felt only in the region of the mass, and these slowly increased in frequency, duration, and severity. The patient thought she had failed

slightly in weight, but she was still well nourished and there had never been other than normal urine in character and amount.

Examination showed a large, hard, smooth, globular and movable but not tender mass in the region of the right kidney.

The tumor with the persisting remnants of the kidney was easily removed through the usual lumbar incision and the patient made an uninterrupted recovery and is quite well at present, *i.e.*, January, 1907.

Macroscopical description.—The specimen, received at the Pathological Department, consisted of the tumor with some preserved kidney tissue. The weight was three hundred and fifty grams; size $9.5 \times 8.5 \times 5.7$ centimeters; the tumor proper was 4.7 centimeters in diameter and showed plainly its separation from the remaining kidney tissue, which consisted of the upper and lower poles. It occupied the center of the convexity and appeared to divide the kidney both longitudinally and transversely; it was underneath the kidney capsule. The ureter was patent and the pelvis, save for being slightly dilated, was unaltered.

Upon section along the convexity the mass was found to be globular, and to extend from immediately under the capsule well into the pelvis, thus occupying and replacing the whole central portion of the kidney. It was well marked off from the kidney tissue but had no definite capsule. This is well seen in the portion projecting into the pelvis.

Toward the center a rather large area of the tumor appeared to consist of ordinary fat, but without trace of lobulation. This shaded off into a more friable zone of a grayish color, which showed considerable reddish-brown mottling, giving the impression that it was stained by extravasated blood. In one place this grayish material was well marked off from the surrounding more yellowish tissue and appeared as a distinct localized nodule which only on one side shaded off gradually into the yellowish areas, as shown in the photograph, but in other places the transition was gradual.

Two small fatty looking masses, about three centimeters in diameter, were present, deep in the kidney tissue of the upper pole, and proved on careful examination to be separate from

the larger tumor. No trace of a capsule could be found here.

Microscopical description. — The tumor may be described briefly as consisting in the main of fat-like tissue, in which are scattered areas of vessels, mostly thick walled and surrounded by what appears on superficial examination to be young fibrous tissue; while in the third place there are found here and there, though mainly in the periphery, small areas of large, irregular-shaped, fat-containing cells of indeterminate type, — cells which we may at the outset call the true tumor cell, forming, as we believe, the parenchyma of the growth.

Taking the sections more in detail, and leaving the more important, that is, the true tumor cell, to the last, we may begin with a detailed description of the fibro-vascular portions. These may be regarded as probably the stroma of the tumor. The elements which go to make it up are, on the one hand, vessels, and on the other, strands of fairly dense but cellular connective tissue. The vessels are arterial and capillary, the former are extremely thick walled and are numerous, and while they are found in spots all through the tumor they are especially frequent at the periphery of the growth. Similar vessels are even seen outside the periphery of the tumor in the almost unaltered kidney tissue, a fact which leads one to consider them as persisting kidney vessels. As a rule the thickening appears to involve mainly the intima, being a true endarteritis. There is frequently seen also a disposition of hyaline material which is found in all degrees, chiefly in the intima, and in it are seen a few fibrous tissue cells of spindle shape. The lumen is often greatly narrowed, sometimes completely obliterated. As to the media and adventitia they also appear to be often hypertrophied, a point, however, which is not easy to prove.

These thick-walled vessels are apt to run in what may be called aggregations, so that, in the cross section, they form islands. Enclosing them there is frequently found a considerable amount of fairly dense but cellular connective tissue, rather young in type than otherwise, — a fibrillar

tissue containing loosely packed spindle-cells running in bands in several directions so as to interlace, and frequently showing longitudinal and cross sections of these bands in the one field. They are not sufficiently numerous nor aggregated in large enough clumps to give the impression of spindle-cell sarcoma, and indeed it is probable that they represent longitudinal and oblique cuts of the vessels. In places it is difficult to be sure that they are not partly composed of smooth muscle fibers. The capillaries are found not only in these islands of thick-walled vessels, but also, and indeed more especially, in the periphery of the tumor, where one gets aggregations of the true tumor cells; they correspond to the ordinary capillary present in new growths.

Near the periphery of the tumor one sees that the vessels are not infrequently somewhat compressed as to their lumina by a growth of tumor cells, which seem to be advancing along the perivascular sheaths. The tumor cells appear to break slightly through the pseudo-capsule of compressed kidney tissue, and, as a matter of fact, to do so mainly along the course of these thick-walled vessels.

In the second place, we may describe the element of fat which forms so great a portion of the tumor. At the outset it may be mentioned that the relation between the fat areas and the true tumor cells seems to be one of transition; the one goes over insensibly into the other. Starting from the center of the tumor we find that the bulk of the tissue here is apparently pure fat; towards the periphery it loses its fatty character to take on gradually the character of the tumor cell mentioned.

Microscopically, the fat is composed, in the main, of typical fat cells, or at any rate, of fat spaces, with the nucleus frequently not appearing in the section. When present the nucleus is often in the center of the cell, though sometimes pressed flat against the periphery. The shape of the cell is more irregular than in a lipoma, perhaps no more so than in subcutaneous tissue, at least not in the center of the fat collections.

At the border line between the typical tumor cell and the

fat, usually as a matter of fact towards the periphery of the growth, one can trace easily the following changes: The cell body enlarges and becomes granular, the nucleus becoming vesicular and a nucleolus making its appearance. This is an indication of commencing fat degeneration, or, to use the more modern and probably less misleading term, fat metamorphosis. The protoplasm shows now the appearance of small droplets; in the next stage these small droplets are replaced by larger ones; all these upon treatment by Sudan III. proving to be fat. At this stage the nucleus may be surrounded by several fat globules; at a later stage the nucleus lies in the center, or at the side of one huge fat globule. In hardened sections one sees the nucleus lying in a large clear space with nothing but a few granules around it, but with no definite cell body. The nucleus then shares in the degenerative process, and so we finally get a large fat globule without a nucleus, sometimes with and sometimes without a mass of granular detritus.

At times several adjoining cells appear to degenerate simultaneously, the cell limiting membrane to become lost, and the several nuclei come together so that we get finally a large space, partly clear, partly filled with granular detritus, and containing as many as fifteen clear vesicular nuclei, giving rise to the so-called giant cell, a cell which is clearly a degenerative product. Finally, towards the center of the tumor, only large fat-containing spaces are met with, separated by a fine, fibrous and vascular stroma. Here, as before, the thick-walled vessels and fibrous bands stand clearly out in the midst of the fat tissue, but never seem to take any part in the formation of the fat. Free fat globules are often seen in the lumina of the vessels.

We would like to call special attention to the fact that many of the fat spaces show the nucleus in the center, and not at the periphery, as is the case in the genuine lipoma; to the still more significant fact that one finds multinucleated or giant fat cells. The importance of these phenomena in diagnosis will be referred to later in the discussion.

The tumor cells proper may now be described. These

are cells slightly varying in size, but upon the whole large; in shape roundish, or polyhedral. They contain usually a somewhat granular protoplasm which stains faintly, and a large, round or oval nucleus, usually with a nucleolus. The resemblance to the "large clear cell" of Albarran is often striking. Occasional mitoses are seen. These cells are found mainly in the periphery of the tumor, presumably the growing portion, and here they are collected frequently into small groups or rows which present more or less of a fasciculated arrangement, in places resembling greatly the zona fasciculata of the adrenal. In this tissue can be found numerous capillaries, and it is seen that as a rule the cells sit directly upon the capillaries. This arrangement is best, or perhaps only, seen at or near the periphery of the tumor. In this fasciculated arrangement we find fine fibrils separating usually small groups of cells, sometimes only two or three and not infrequently only one, so that we get in places a picture not unlike sarcoma. This fasciculated arrangement is well brought out in sections stained by Mallory's method for connective tissue.

In the area occupied chiefly by these tumor cells, that is, at the edge of the tumor and close to the kidney border, one can find a few isolated kidney tubules, easily distinguished by their smaller size, darker staining and tubular arrangement, from the tumor cells. From their appearance they are collecting, not convoluted, tubules.

It may be remarked in passing that the tumor cell has to some extent a resemblance to the cell of the convoluted tubules of the kidney, found just outside the periphery of the growth; a resemblance which is seen both in size and in the appearance of the protoplasm and nucleus. Especially is this so when the cell of the convoluted tubule shows granular degeneration. Yet it is plainly impossible that the former should have arisen from the latter; microscopically, no continuous connection can be anywhere demonstrated.

In the islands of fibro-vascular type, upon close examination, it is possible to find, not infrequently, small groups of cells which are quite distinct from the drawn-out spindle-cell

of the fibrous type, and which, in short, resemble very markedly the true tumor cell. One also finds in the same areas small collections of fat cells; the relation between the tumor cell and the fat cell in these areas cannot be clearly followed as it can be at the periphery of the tumor.

The tumor, it is true, macroscopically gives the appearance of being separated from the kidney tissue proper by a thin capsule; that is, there is a distinct line of demarcation over nearly the whole periphery between the tumor and the kidney. Yet when one comes to make a microscopical examination, it is plain that the capsule is purely what one might call a compression capsule due to the condensation and slight fibrosis of the adjacent kidney tissue. There is no true fibrous capsule. Not only so but one finds here and there spots where the tumor seems to have invaded the kidney substance to a slight degree. Notably is this the case in some places where kidney tubules, apparently of the collecting type, are found, as above mentioned, isolated inside the tumor capsule, lying amongst the tumor cells.

Sections of the tissue of the two smaller growths show upon the whole the same appearances as these of the main mass. Towards the center of these small masses there are fairly typical fat cells, while at the periphery and directly continuous with the kidney tissue one finds polyhedral epithelial cells and between these two extremes one finds cells showing all grades of fat infiltration. So here we have undoubtedly tissue of the same type as that of the large growth and in strong probability these smaller masses are metastases or else independent growths of the same nature.

The question arises whether the tumor described is malignant or benign. Certain points speak more or less in favor of at least some degree of malignancy. There is a lack of any true capsule; there are the evidences of invasion at the periphery, as previously described, especially seen in the presence of isolated collecting tubules found inside the growth at the periphery, and there are finally the two minute fatty nodules lying apart from the main tumor in the kidney tissue, which under one interpretation may be regarded as local

metastases rather than as independent new growths, inasmuch as they show, upon the whole, a microscopical structure similar to that of the main tumor.

On the other hand, the general appearance of the tumor and the clinical course, speak strongly for its benign nature. We may with probability regard it as being on the borderline between the two conditions.

DISCUSSION. — The diagnosis of the tumor above reported is a very difficult one. As we have seen, the growth possesses many points of similarity to other fatty tumors of the kidney, and, on the other hand, certain points of dissimilarity from them. In endeavoring to make the differential diagnosis, the discussion may be carried out along two lines: First, that which, starting from the fat as the chief element, attempts to trace its origin; and second, that which, considering the tumor cell as the principal element, endeavors to find its genesis.

Perhaps it will be best, first of all, to compare briefly our tumor with those which have been hitherto described, the analysis of which appeared at the beginning of this article, after which we may proceed to consider the tumor on its own merits, and to endeavor to give it "a habitation and a name."

In the first place, the fatty growths that Ulrich describes cannot properly be compared with the present one. His were all small, and were definitely (as his pictures show convincingly enough) pure degeneration products from kidney cells. It is inconceivable that a mass of tissue consisting of fat, hypertrophied vessels and tumor cells, such as our tumor presents, should have been derived from any such origin.

In the second place, we have those tumors which have been called pure lipoma. In so far as these have been made out to consist of fat alone they have been all extremely small, a point which at once differentiates them from our own; the larger ones, those which have been described by Alsberg, Warthin, Bartsch and Grawitz, contained nowhere, according to the published results, any collections of cells

which could suggest an epithelial or sarcomatous origin, or, on the other hand, an adrenal growth. Alsberg's were multiple nodules, each of which might be taken as pure lipoma, but the largest of which was really very small. Warthin's was a very large growth but was composed only of fibrous tissue and fat, a fibro-lipoma. In the case of Bartsch and Grawitz, there were present not only fat, fibrous tissue and angiomatous tissue, but also some muscle; they, however, belong rather to the class of Mueller's "Mixed fatty tumors of the kidney," than to the class of pure lipoma. Mueller terms his cases lipo-myo-sarcoma, calling them mixed tumors on account of there being present muscle as well as fat and fibrosarcomatous tissue.

Mueller's theory as to the histogenesis of his "lipomatöse Mischgeschwülste der Niere" was that they arose in misplaced bits of the kidney capsule; they represented a coincident development of three separate and independent forms of tissue—fat, muscle, and fibrous or sarcomatous tissue, which had been herniated into the renal substance from the renal albuginea,—a dislocation which occurred during embryonic life after the fashion of von Recklinghausen's adenomyoma of the uterus. It is hardly here the place to enter upon the discussion of this point; nevertheless, if the metamorphosis of a misplaced adrenal rest into true fat be accepted, as is suggested by the tumor here described, one is justified in speculating whether some at least of Mueller's cases, and cases 13 and 14 of Horn's list, were not in reality hypernephroma with fatty change so as to simulate lipoma. The presence of muscle speaks as much for an adrenal as for a renal origin; indeed, the amount of smooth muscle fibers in the adrenal capsule is greater normally than in the renal capsule. The same doubt, though confessedly improbable, is perhaps permissible with regard to the instances of coincident and contiguous development of hypernephroma and lipoma described by Alsberg, Horn, and Grawitz.

Our tumor, however, differs from this class in containing no muscle, and secondly, in the presence of the cells which

we have called the true tumor cell, which, although comparatively small in numbers, seems to form in our growth the true parenchyma.

In the fourth place we must differentiate this growth from the typical hypernephroma. First of all, any resemblance to the now well-established typical picture of hypernephroma is, in the microscopical section, quite lacking. Although containing much fat, it is not at first sight a pseudo-lipoma in Grawitz's sense. On dissolving out the fat with alcohol, the well-known matrix of large clear cells does not appear; on the contrary, we find at first only pure fat and the fibro-angiomatic tissue. When we search more closely, however, we find in our tumor certain cellular areas, as above described, which, it may now be said, give us the general impression of being of adrenal origin because of their architectural arrangement and their fatty contents. Of pseudo-lipoma, or hypernephroma, there are now reported a great many typical cases, which it is unnecessary to review in this place. From these our tumor differs in certain points: first, the presence of apparently genuine, fully formed fat tissue, as opposed to fatty infiltration of adrenal cells, to such a degree indeed as to simulate true lipoma; in the second place, the comparatively small number of these cells which suggest an adrenal origin, compared with their numbers in the typical hypernephroma.

If then there are in our growth certain points which indicate to us a possible origin from the adrenal, while true fat is the main element of the mass, we are obliged to consider a fifth possibility and to differentiate this growth from those in which there are present hypernephroma and lipoma side by side, contiguous yet independent, such as was described in one of Mueller's cases and in one of Horn's cases, and in one or two others. In these, the two tissues, so far as one can judge from the published descriptions and from the illustrations (which as a matter of fact are sadly lacking), are quite independent, do not invade each other, and show no transition pictures.

In these very points do we find a striking dissimilarity

from our tumor. In the mutual invasion, or rather in the innumerable transition pictures which one finds from the tumor cell to the fat cell, we have the chief point which distinguishes this tumor from typical hypernephroma on the one hand, pure lipoma on the other, and, in the third place, from an independent coincidence of the two. We therefore feel justified, having carried the discussion thus far, in suggesting that the present growth represents in all probability a hypernephroma which in the course of development has taken on a new variation of growth, that of a metamorphosis into fat — fat which is frequently indistinguishable from the true fat of a lipoma or of subcutaneous tissue. That such a condition has heretofore been described, or even plainly suggested, is to us at least unknown.

Resuming the discussion along the second line above indicated, that which considers the tumor cell in itself and endeavors to trace its genesis from its intrinsic characters, one is obliged at the outset to admit that such an attempt must naturally remain somewhat unsatisfactory. On the one hand the close relationship which it bears to the vessel walls and the presence of the pericellular reticulum, which is often present, suggests a mesoblastic origin, while the well outlined cell with deeply-staining nucleus, occasionally occurring in groups surrounded by a fibrous stroma, and in places infiltrating the perivascular lymph spaces, recalls the appearance and mode of growth of epithelial tumors.

This picture, then, in which the individual cell reminds one of the epitheliomatous type of growth, while the relation of cells to reticulum and vessels suggests the type of tumor arising in mesoblastic tissue, — this picture is what we have come to look upon as being best described by the term endothelioma.

Now, a study of the kidney tumors since Grawitz shows us how intimately connected are the cells of the adrenal cortex with endothelium. The adrenal cortex, as has been shown, develops as a perithelioma, and Stilling¹⁸ has proved that the lumen which is found between the adrenal cell groups in the cortex is in reality a lymph space. Thus we

have some justification for assuming that the origin of this tumor lies either in endothelium or in cells of the adrenal cortex; if the latter, then it is, in other words, a true hypernephroma.

We are strongly inclined to believe that the cell comes from the latter of these, that is, the adrenal, in view of the following facts: the essential disposition to fat metamorphosis; the morphology of the individual cell; the fasciculated arrangement of the cell groups, recalling strongly the architecture of the adrenal cortex; the overgrowth of the intima of the vessels and the disposition of hyaline material in them (so often met with in hypernephroma); the way in which the tumor cells sit directly upon the capillaries; and, upon the macroscopical side, the situation of the tumor underneath the kidney capsule. Finally, the fact that there is some evidence showing a mild degree of malignancy, with local metastases, would also point to some extent towards hypernephroma.

The crucial point of the question along general lines lies in the interpretation of the genesis of the fat. Virchow's theory being here untenable, inasmuch as the tumor cells are plainly not fibroblastic, there remain only two other theories by which we may account for it: First, that it is a growth from preëxisting fat, that is, that it represents an inclusion from the kidney capsule. This theory is here contradicted by the presence of the transition pictures leading from the fat to a tumor cell. At the best, by this theory, one would have to assume a simultaneous inclusion of adrenal and capsule fat—the reasons against this view have already been given. In the second place, the fat might be presumed to come from an overgrowth of the adrenal tissue itself, that is, from an adrenal rest. Although the theory that the adrenal cell in hypernephroma, such as we ordinarily see it, should form by metamorphosis a true fat cell may seem on the surface unlikely, yet it must be remembered that there exist certain analogies. If we assume, with Virchow, that fat ordinarily is derived by a sort of infiltrating process from a fibroblastic cell—that is ultimately from a mesothelial cell—what

objection can there be to supposing that a different variety of the mesothelial cell, in this instance the adrenal cell, should also take up fat and become morphologically a true fat cell? Such a thing in reality does occur, at least we have the authority of Albarran¹⁴ that the fat in the adrenal normally is not infrequently collected into fatty nodules, comparable with the fatty nodule condition of the liver already known.* And again, we have Ulrich's demonstration that small areas of the convoluted tubules of the kidney may undergo fat degeneration or fat metamorphosis to such an extent as to simulate lipoma.

There is, therefore, perhaps, no essential impossibility, or even difficulty, in assuming theoretically that the adrenal cell may become transformed into a fat cell, provided we accept, as is now generally admitted, a mesothelial origin for the cortex of the adrenal. And even if it were contended that the adrenal cortex was epiblastic in origin, it may be fairly questioned whether that would, of itself, be sufficient to kill the theory; it has not yet been proven that an epiblastic cell cannot turn into a fat cell; and the fatty nodule condition of the liver described by Lubarsch might be adduced as evidence to the contrary.

We have not considered it necessary to go in detail into the minute and more doubtful evidences which have been fought over concerning the diagnosis of hypernephroma. Glycogen, for instance, was not found in our tumor; but it must be confessed that only a very cursory examination was made for it, inasmuch as the growth at first sight suggested anything but hypernephroma. The value of this test is, however, admittedly slight. Glycogen is the sign of rapid growth, and may be found, as Bra has shown, in practically all malignant tumors. Its absence in what was plainly a very slow-growing tumor, one which tended rather to degeneration

* He quotes Letulle's note to the Société Anatomique of Paris, in 1889, which we here transcribe: "La surcharge grasseuse (of the adrenal) chez l'homme n'est pas constante, et se trouve surtout chez des sujets ayant succombé avec des troubles de l'appareil circulatoire. Elle est souvent irrégulière, respectant sans raison apparente des fragments étendus de trabecules capsulaires. Elle se forme souvent en nodules graisseuses analogues à l'état nodulaire graisseux du foie."

or metamorphosis than to rapid proliferation, can hardly be cited as evidence against the theory here propounded.

Lecithin was not looked for.

The dispute as to the differential diagnosis between hypernephroma and endothelioma, or, as Hanseemann prefers to call it, adenoma endotheliale, does not concern us. It is, after all, largely a question of terms. The adrenal itself is in a way an endothelioma or endothelial adenoma. In our own case it is clearly impossible to say certainly that the tumor is not an adenoma, although the reasons given point in our opinion strongly to the adrenal origin. The main thing is that, which ever it is, true fat seems to have been developed out of it in large quantity.

[In conclusion we desire to acknowledge our indebtedness to both Prof. James Bell, in whose service the case occurred, and Professor Adami, who kindly placed the material at our disposal for investigation.]

MONTREAL, Jan. 7, 1907.

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DESCRIPTION OF PLATES X. AND XI.

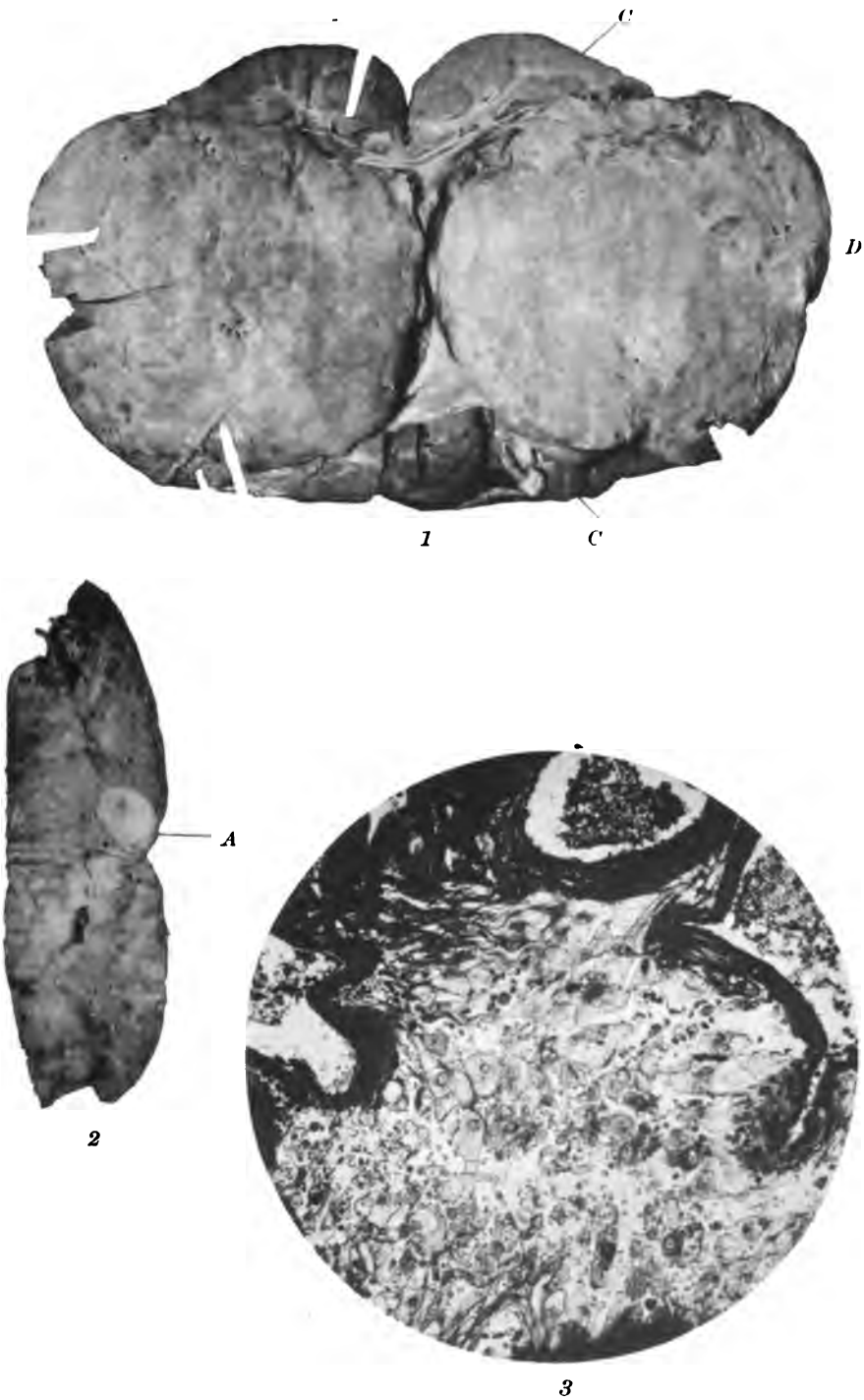
FIG. I. — Kidney sectioned along convex border into pelvis showing (D) the globular tumor replacing the central portion of the kidney and (C) the upper and the lower poles of the kidney remaining.

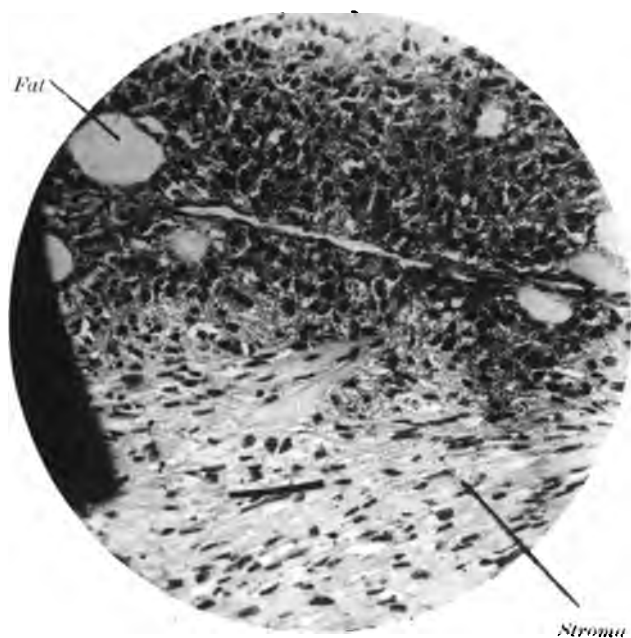
FIG. II. — A portion of the tumor showing the grayish friable nodule "A."

FIG. III. — Shows the large clear cells, the multinucleated cells, the thick walled vessels and the extravasated blood (eye-piece 3, objective 7, "Winckel").

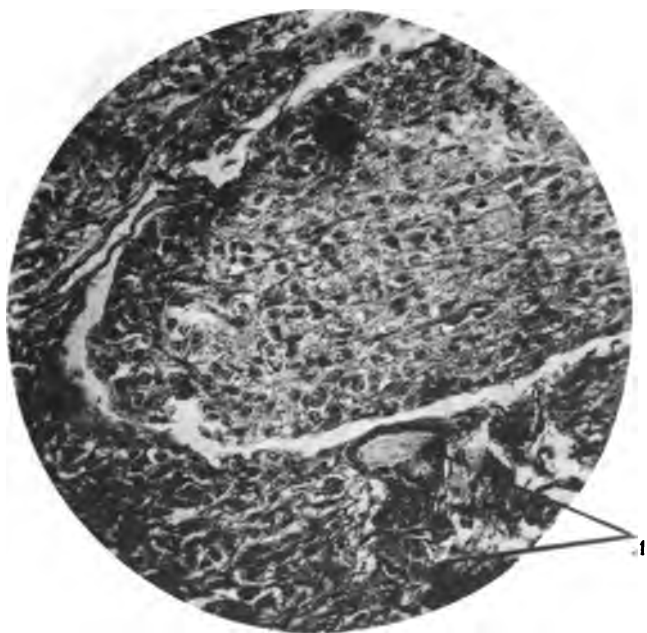
FIG. IV. — Shows a capillary surrounded by the tumor cells, also a few fat spaces and at one side a stroma band (eye-piece 3, objective 7, "Winckel").

FIG. V. — Shows the periphery of the growth with (A) two enclosed kidney tubules. It shows also the columnar arrangement and the pericellular reticulum.





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THE
Journal of Medical Research.

(NEW SERIES, VOLUME XI.)

VOL. XVI.

MAY, 1907.

No. 2.

ON SERUM ANAPHYLAXIS IN THE GUINEA-PIG.*

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Animals may react to certain toxic or foreign substances in one of two ways, either by an increased resistance or immunity, or by an increased susceptibility or anaphylaxis.† The reactions of immunity have been demonstrated not only by the increased resistance of the treated animal but also by the occurrence of specific anti-bodies in its blood. Far less is known of the nature of anaphylaxis. Our conceptions of immunity and anaphylaxis presuppose that certain changes have taken place in the somatic cells; theories of animal reaction have been founded on such supposed cell changes, although we possess, in reality, no definite information as to their nature. The present study brings out the mechanism of a striking instance of anaphylaxis and throws light on a definite cell pathology consequent upon the general condition of increased susceptibility.

Attention has been drawn by Otto² to a remarkable toxic phenomenon in guinea-pigs. Otto's work was carried out in

* This study has been facilitated by a grant from the Proctor Fund of Harvard University. Received for publication March 28, 1907.

† This word was first used by Richet¹ to designate the increased susceptibility to a poison from Actinians shown by dogs and rabbits which had survived a small dose of this toxic substance.

the Serum Institute at Frankfort, Germany, following an oral communication to Ehrlich on the part of Theobald Smith. Rosenau and Anderson,³ working independently of Otto, brought out shortly afterward an extensive monograph which confirmed and elaborated the findings of the German investigator. Circumscribed and definite as the phenomenon appears, it is not easy to follow its evolution. The reaction, as shown by the work of Otto and of Rosenau and Anderson, is somewhat as follows: The injection of any convenient amount of horse serum subcutaneously or intraperitoneally in normal guinea-pigs produces no ill effect. If, however, guinea-pigs are given a very small dose of horse serum, *e.g.*, .0001 to .1 cubic centimeter, and ten days or two weeks are allowed to elapse, a second injection of a relatively large amount, *e.g.*, five cubic centimeters, will cause violent and characteristic symptoms, followed almost invariably by death within the hour. This reaction to a second large dose of horse serum was first noted in guinea-pigs which had survived a diphtheria toxin-antitoxin mixture; but the acquired susceptibility to the serum is in no way dependent on the presence of diphtheria toxin in the first, or "sensitizing," dose, but on the small amount of horse serum which contains the antitoxin.

The literature of immunity bristles with instances of an increased specific susceptibility in animals which have resisted doses of toxic or even apparently non-toxic substances. A list of many such phenomena, more or less closely allied to this serum disease of guinea-pigs, has been given both by Otto and by Rosenau and Anderson. It is of interest to note that explanations of this hypersensitivity are almost as numerous as the instances which have been described; in certain well-studied cases numerous theories exist; for example, there are at least five well-maintained hypotheses concerning the toxic effect of tuberculin in tuberculous animals. The serum disease of guinea-pigs, which we are now considering, apart from its intrinsic interest, offers particularly advantageous conditions for the study of a reaction in the animal body which we believe has not as yet

been recognized, and which would appear to be of considerable importance.

In a preliminary consideration of this serum intoxication phenomenon under the simplest conditions, it will be necessary to restate briefly some of the factors which have already been noted by the observers who have preceded us. In fact, in the first phase of this study, only one new fact stands out, namely, an almost constant finding of definite lesions, which lesions had been overlooked both by Otto and by Rosenau and Anderson. In our experiments young normal guinea-pigs have been used which weighed at the beginning of treatment from one hundred and fifty to two hundred and fifty grams. The serum both for sensitizing and for toxic effect has been from normal horses, and has been taken aseptically, preserved in sterile bottles without the addition of an antiseptic, and kept at 0° C. until used. It was found by Rosenau and Anderson that a dose of horse serum so small as .00001 or even in one instance .000001 of a cubic centimeter sufficed to render guinea-pigs sensitive to the subsequent toxic dose of serum; it has not concerned us to define so precisely the minimal dose necessary, but we have used in nearly all our experiments an arbitrarily chosen dose of .01 cubic centimeter administered subcutaneously or intraperitoneally. As a period of incubation we have found it better to wait at least the fourteen or fifteen days advised by Otto. The animals remain sensitive for an undetermined period, at least for many months. For the second toxic dose, five cubic centimeters of horse serum intraperitoneally are necessary to cause death, although much smaller doses will produce characteristically severe symptoms; when injected subcutaneously a considerably larger dose is necessary.

An animal treated as has been outlined, on receiving the second dose of serum intraperitoneally, shows some or all of the following symptoms, and dies, in the majority of the cases, within an hour. In from two to fifteen minutes after injection may be noted twitching of the ears or body, coughing, retching, and less frequently general muscular

contraction, with "bucking" and rushing about the cage; after the period of irritation, paralytic symptoms intervene, the animal squats, or feebly drags itself about the cage, complains querulously, shows respiratory distress, and finally falls on its side. This comatose period may last for some time until death intervenes, or the exitus may be precipitated by violent general spasms. The heart beats for some time after the cessation of respiration. Either the irritative or the paralytic symptoms may predominate. An animal may recover after having passed through most characteristically severe phases of both. Death is not a necessary criterion of the complete evolution of the disease, or of the presence of the characteristic lesions; severe symptoms, however, whether followed by death or recovery, are almost invariably accompanied by definite macroscopic findings at autopsy.

Hemorrhage, rather definitely localized, is the characteristic gross lesion in this acute disease. The hemorrhages may be found in one or in several organs; gastric hemorrhage occurs in the majority of cases. The summary which follows indicates the percentage of cases in which lesions occur, and the localization of these lesions. Forty-one guinea-pigs which were examined seriatim fall into two categories: those pigs which died as the result of the disease, and those animals which after having suffered severe and characteristic symptoms were on the road to recovery, or had fully recovered, and were chloroformed within twenty-four hours.

1. Guinea-pigs which died of serum intoxication, twenty-nine animals. Hemorrhage present in twenty-three cases; hemorrhage absent in six cases.
2. Guinea-pigs chloroformed within twenty-four hours after severe symptoms, twelve animals. Hemorrhages present in eleven cases; hemorrhages absent in one case.

Gastric hemorrhages were present in thirty-two of the forty-one cases. Hemorrhages were localized in other organs as follows: cecum in ten cases; lungs, nine cases; spleen, seven cases; adrenal, six cases; heart, three cases;

pericardium, two cases; diaphragm, two cases; lymph glands, two cases; liver, kidney, thyroid, and muscle, each one case.

As has been noted, severe symptoms, irrespective of the subsequent occurrence of death, are sufficient to indicate the evolution of a disease accompanied by definite hemorrhages. It is seen that gastric hemorrhage is a most common but not necessarily constant finding in the well-marked disease. A list of the control animals which have been used to limit these lesions to the disease entity will be given in connection with the microscopic study. It should be noted in this place, however, that the occurrence of hemorrhage in the abdominal organs is not due to the fact that most of the injections were intraperitoneal since the same results are obtained by subcutaneous injections. A detailed description of the macroscopic appearances of these hemorrhages, apart from the gastric and cecal lesions, is hardly necessary; they are minute in general, rather larger in the lungs and spleen than elsewhere. In the stomach the localization is of importance; the lesions which may be one, or two, or multiple, vary in size from a pin point to one or two centimeters in diameter and occur characteristically midway in the greater curvature or in lines diverging from this point, either toward the extremities of the viscus or encircling it toward the middle point of the lesser curvature; they do not occur at the pylorus, in our experience, and are therefore to be differentiated from the lesions described recently by Rosenau and Anderson⁴ as caused by diphtheria toxin. In milder grades of lesion the hemorrhages are merely submucous; but in the more advanced stages there is definite erosion, and the stomach may contain clots of blood. The cecal hemorrhages occur about or in the agminated follicles.

We do not wish to insist on the gastric hemorrhages, which occur so frequently in this disease, as anything more than the localized manifestation of a general reaction. They are of interest as stomach lesions produced experimentally without direct injury, and, when studied more minutely, throw light on the evolution of the disease itself.

When we examine more attentively the factors necessary to produce this striking and acute disease the two most salient points are the extremely small dose necessary to sensitize and the existence of a period of incubation. It would be noted by the most casual observer that this incubation period of from ten to fifteen days corresponds to a nicety to the period necessary for the maximal production of an anti-toxin after the injection of a toxin, or of a cytolsin after injection of cellular elements. The surprisingly small amount necessary to sensitize need not preclude the formation of anti-bodies during this time, for analogous instances of minute doses of blood leading to the production of a hemolysin are on record. And it is perhaps this corresponding incubation period, more than any other factor, which has led Rosenau and Anderson to a tentative but, as it would seem, incorrect explanation of this phenomenon. These authors believe that the toxic reaction caused by the second dose of serum is due to an interaction between anti-bodies which have been formed against the sensitizing dose and certain toxic substances present in horse serum; they have further assumed, on no certain experimental grounds, that the sensitizing and the toxic substance in horse serum are identical, an assumption in reality necessary to maintain their interaction hypothesis.

Otto has noted that a large initial dose does not cause the sensitivity which regularly follows the usual small dose. Similar results have been indicated by the experiments of Rosenau and Anderson. To these authors such a finding would correspond to other experiments which in their hands seemed to them to prove that repeated doses of serum at short intervals give rise to an immunity. That is, a small amount of horse serum sensitizes guinea-pigs, a large amount, in a single or multiple dose, seems to immunize. If one experiments only so far as Rosenau and Anderson and is dominated by classical examples of the formation of anti-bodies, such a conclusion might be drawn; but if we consider such experiments more exhaustively, a wholly different conclusion emerges. It may be shown, as is evidenced by

the following experiment, that a single large initial dose gives rise, not to immunity, but to a temporary insusceptibility which is later followed by characteristic sensitivity.

Experiment 1. — Normal guinea-pigs of similar weight were separated into two lots and injected intraperitoneally with:

Lot A, 8 cubic centimeters of horse serum.

Lot B, .01 cubic centimeter of horse serum.

The comparative susceptibility of these pigs to a toxic dose of horse serum (5 cubic centimeters) was tested at the following periods:

After 20 days: A pig, No. 1, weight, 365. Slight nervous symptoms.

After 20 days: A pig, No. 2, weight, 385. Slight nervous symptoms.

After 20 days: B pig, No. 1, weight, 380. Dead, 24 minutes.
Diaphragmatic and gastric hemorrhages.

After 20 days: B pig, No. 2, weight, 300. Dead, 20 minutes.
Small gastric hemorrhages, hemopericardium.

After 36 days: A pig, No. 3, weight, 385. Marked symptoms for four hours. Recovered.

After 45 days: A pig, No. 4, weight, 485. Dead, 40 minutes.
Hemorrhage into stomach and lungs.

After 45 days: A pig, No. 5, weight, 395. Dead, 45 minutes.
Hemorrhage into stomach.

The conclusion from such an experiment may, for the moment, be stated simply as follows: the injection of a large initial dose of horse serum delays the period of incubation necessary to produce sensitivity beyond the period when a small dose is employed.

As has been stated, Rosenau and Anderson have shown that repeated injections of moderate doses of horse serum into guinea-pigs at short intervals are followed by no reaction and by an apparent state of immunity or resistance to the toxic dose of serum. Their own experiments should have pointed to the possibility of a different conclusion. As a matter of fact, if a good sized injection (two to five cubic centimeters) is repeated every few days for a few times and then two weeks or even longer allowed to elapse, the subsequent injection of a dose that would cause severe symptoms or death in a properly "sensitized" pig (*i.e.*, one having received a single small initial dose) produces no symptoms. If, however, one waits several weeks, such apparently

"immune" pigs become sensitive to reinjection, the degree of sensitivity varying directly with the time elapsed since the last injection and with the total amount of serum previously injected.

The histories of the following guinea-pigs show the absence of reaction to repeated doses of horse serum given at short intervals. These animals were injected with from two to five cubic centimeters of horse serum at intervals, which, in days, are represented by the numerals.

- G.P. A. Injected at intervals of 1, 2, 2, and 2 days. No symptoms.
- G.P. B. Injected at intervals of 1, 2, 2, and 2 days. No symptoms.
- G.P. C. Injected at intervals of 1, 2, 2, 2, and 13 days. No symptoms.
- G.P. No. 1. Injected at intervals of 10, 9, 13, 4, and 4 days. No symptoms.
- G.P. No. 2. Injected at intervals of 2, 9, 10, 4, and 4 days. No symptoms.
- G.P. No. 3. Injected at intervals of 9, 9, 13, 4, and 4 days. No symptoms.
- G.P. No. 4. Injected at intervals of 9, 9, and 13 days. No symptoms.
- G.P. No. 5. Injected at intervals of 2, 9, and 13 days. No symptoms.

When, however, some of the above animals were kept for longer intervals after the treatment recorded and then injected, the following results were obtained:

- G.P. No. 3. Injected 38 days after treatment above. Distinct symptoms.
- G.P. C. Injected 48 days after treatment above. Slight symptoms.
- G.P. No. 4. Injected 55 days after treatment above. Very severe symptoms. Recovered.
- G.P. No. 2. Injected 82 days after treatment above. Very severe symptoms. Recovered. G.P. No. 2 was chloroformed after 4 hours continuous symptoms, and at autopsy typical gastric hemorrhages were found.

Animals that have received one large dose or multiple doses may be referred to as "refractory," and that such refractory animals are at no period really immune to horse serum may be shown in several ways. This condition of

eventual susceptibility might be thought to be allied to such conditions as the susceptibility of certain diphtheria or tetanus antitoxin animals to relatively small doses of their respective toxins; such animals present the curious paradox of free antitoxin in the blood and diminished resistance of the tissues. The case is not analogous because these refractory guinea-pigs do not produce an antitoxin to the toxic substance in horse serum.

Experiment 2. — Guinea-pig No. 5, which had received five injections of horse serum (total, 12 cubic centimeters), was bled ten days after the last injection. A normal guinea-pig was also bled. To nine cubic centimeters of the respective sera, No. 5 and normal, was added five cubic centimeters of horse serum, and the mixtures left over night at room temperature. There was a distinct precipitate in the G.P. No. 5 + horse serum mixture which was removed by centrifuging. Guinea-pigs, sensitized by the injection of .1 cubic centimeter horse serum forty days previously, were injected with the mixtures:

G.P. No. 8, weight, 585, given mixture (Serum G.P. No. 5 + horse serum).

G.P. No. 6, weight, 625, given mixture (Serum Normal + horse serum).

G.P. No. 8. Dead in 45 minutes (gastric and cecal hemorrhages).

G.P. No. 6. Very severe symptoms; recovering 5 hours; chloroformed. (Gastric hemorrhages.)

Repetitions of such an experiment always show that the serum of a guinea-pig which has received several injections of horse serum and has been bled at the critical antitoxin period (and also at a period when the animal is resistant to the toxic effect of horse serum) contains no anti-bodies which neutralize the toxic substance in horse serum. Such refractory guinea-pigs frequently show at this period precipitins for horse serum.

Although the blood of refractory animals contains no anti-body for the toxic element of horse serum, we might at least imagine that the sensitizing substance of the serum is neutralized in the body. We might even imagine that such guinea-pig serum contains an anti-sensitizing substance; this finding, however, would be difficult to explain in view of the eventual sensitization of the refractory pigs. In fact we find that the

serum of refractory pigs, at a period when the animals are resistant to horse serum, not only does not contain anti-sensitizing substances, but *actually contains that substance of horse serum which sensitizes guinea-pigs.*

Experiment 3.—Normal guinea-pigs of similar weight were injected subcutaneously with the same amount of serum of other guinea-pigs which had been treated as follows:

G.P. No. 53. Given subcutaneously 1.5 cubic centimeters serum normal guinea-pig "X."

G.P. No. 75. Given subcutaneously 1.5 cubic centimeters serum normal guinea-pig "Y."

G.P. No. 76. Given subcutaneously 1.5 cubic centimeters serum normal guinea-pig "Z."

G.P. No. 82. Given subcutaneously 1.5 cubic centimeters serum refractory pig "A."

"A" was bled 10 days after receiving the last of 5 injections horse serum. (Total, 13 cubic centimeters.)

G.P. No. 86. Given subcutaneously 1.5 cubic centimeters serum refractory pig No. 4.

No. 4 was bled 19 days after the last of 5 injections horse serum. (Total, 27 cubic centimeters.)

G.P. No. 50. Given subcutaneously 1.5 cubic centimeters serum refractory pig "B."

"B" was bled 11 days after the last of 5 injections horse serum. (Total, 9 cubic centimeters.)

G.P. No. 51. Given subcutaneously 1.5 cubic centimeters serum refractory pig "C."

"C" was bled 14 days after the last of 7 injections horse serum. (Total, 17 cubic centimeters.)

G.P. No. 54. Given subcutaneously 1.5 cubic centimeters serum refractory pig No. 1.

No. 1 was bled 9 days after the last of 6 injections horse serum. (Total, 16 cubic centimeters.)

After 15 days these pigs were given each 5 cubic centimeters of horse serum into the peritoneal cavity with the following results:

G.P. No. 53, No. 75, No. 76. (Controls.) No symptoms.

G.P. No. 82. Severe symptoms. Recovering 2½ hours. Chloroformed. Gastric hemorrhage

G.P. No. 86. Marked symptoms. Recovered.

G.P. No. 50. Severe symptoms. Recovered.

G.P. No. 51. Dead in 90 minutes. (Diaphragmatic and splenic hemorrhages.)

G.P. No. 54. Dead in 45 minutes. (Gastric and renal hemorrhages.)

There is further evidence that guinea-pigs refractory to horse serum still contain the sensitizing substance of the serum unneutralized. The offspring of refractory animals, born at a period when the mother is resistant to the toxic action of horse serum, *are sensitive, not resistant*.

1. Guinea-pig "C," two days after the sixth injection of horse serum (total, 12 cubic centimeters), had one young (No. 26). When six weeks old this offspring was given five cubic centimeters horse serum intraperitoneally. Very severe symptoms. Recovered. Chloroformed after eighteen hours. Two hemorrhagic erosions of stomach.

2. Guinea-pig No. 4, three days after receiving last of four injections of horse serum (total, 17 cubic centimeters), had three young. Of these offspring two were injected as follows:

No. 42. (Seven weeks old), five cubic centimeters horse serum *subcutaneously*. Severe symptoms. Recovering one hour. Chloroformed. Several small gastric hemorrhages.

No. 43. (Nine weeks old), five cubic centimeters horse serum intraperitoneally. Very severe symptoms. Recovering two and one-half hours. Chloroformed. Pin-point hemorrhages of cecum. Small gastric hemorrhages.

There is an analogy between these last experiments on the transmission of sensitivity to offspring from refractory mothers and certain experiments of Rosenau and Anderson⁵ and of Anderson;⁶ these authors found that the young of sensitized guinea-pigs were also sensitive to horse serum. It was only a step further for us to demonstrate that normal pigs may be rendered sensitive to horse serum by injecting them with the serum of sensitive pigs and awaiting the proper incubation.

Experiment 4.—This experiment was carried on synchronously with Experiment 3, and they therefore supplement and control one another. Normal guinea-pigs were injected subcutaneously with the same amount of sera of other guinea-pigs treated as follows:

G.P. No. 48. Given 1.5 cubic centimeters serum of sensitized pig No. 14. (No. 14 was bled 16 days after receiving .01 cubic centimeter horse serum.)

G.P. No. 49. Given 1.5 cubic centimeters serum of sensitized pig No. 33. (No. 33 was bled 14 days after receiving .01 cubic centimeter horse serum.)

G.P. No. 77. Given 1.5 cubic centimeters serum of sensitized pig No. 61. (No. 61 was bled 29 days after receiving .01 cubic centimeter horse serum:)

The actual sensitivity of these pigs, 14, 33, and 61, was controlled by injecting parallel animals, which had been sensitized the same day, with a toxic dose of horse serum within 24 hours of the time of bleeding their fellows. In each control death occurred in the characteristic manner.

After fifteen days pigs 48, 49, and 77 received each 5 cubic centimeters horse serum intraperitoneally with the following results:

G.P. No. 48, slight symptoms.

G.P. No. 49, severe symptoms.

G.P. No. 77, symptoms. Chloroformed after four hours. No gastric hemorrhage.

It would seem sufficiently astounding that 1.5 cubic centimeters of the serum of a guinea-pig which had received .01 cubic centimeter of horse serum twenty-nine days previously contains sufficient horse sensitizing substance to sensitize a normal guinea-pig to the toxic action of horse serum; the question, however, properly arises whether such guinea-pigs, which remain sensitized so long, uniformly contain substances in their blood which will sensitize normal pigs. An experiment directly bearing on this question follows:

Experiment 5. — There came into our hands, through the courtesy of Dr. P. A. Lewis, three guinea-pigs which had been treated several months previously with a toxin-antitoxin mixture. These animals had the following histories as regards previous injection of horse serum (diphtheria antitoxin).

G.P. No. 4446. Received .005 antitoxin 204 days previously.

G.P. No. 4452. Received .003 antitoxin 204 days previously.

G.P. No. 4583. Received .01 antitoxin 169 days previously.

These animals were bled for a small amount from the carotid and a few hours later two of them were injected into the peritoneal cavity each with 5 cubic centimeters of horse serum, to prove their sensitivity. The results of these injections were:

G.P. No. 4446. Dead in 35 minutes. Gastric hemorrhages.

G.P. No. 4583. Dead in 55 minutes. Small gastric hemorrhages.

Normal guinea-pigs were injected as follows:

G.P. No. 108. Given subcutaneously 1.5 cubic centimeters serum from G.P. No. 4452.

G.P. No. 109. Given subcutaneously 2.5 cubic centimeters serum from G.P. No. 4446.

G.P. No. 110. Given subcutaneously 2.5 cubic centimeters serum from G.P. No. 4583.

Fifteen days later these pigs were given 5 cubic centimeters horse serum into the peritoneal cavity with the following results:

G.P. No. 108. Dead in 12 minutes. Hemorrhage of spleen and lungs.

G.P. No. 109. Dead in 60 minutes. Hemorrhage of stomach and lungs.

G.P. No. 110. Dead in 12 minutes. Hemorrhage of the lungs.

It is evident from these experiments that sensitivity in guinea-pigs treated with horse serum is accompanied by the constant presence of a substance in their blood which will sensitize other guinea-pigs to the toxic action of horse serum. It would not seem necessary to assume that this sensitizing substance, in the serum of sensitized pigs, is other than the sensitizing substance of horse serum, which has remained unneutralized in the guinea-pig body. The resistance of this substance for many months in a foreign organism is surprising, but diminution might occur to a marked extent and still leave enough to sensitize a normal pig; Rosenau and Anderson have noted that only .000001 cubic centimeter is necessary to produce sensitivity in the first place. Sensitivity would appear to depend upon the presence of this unneutralized substance of horse serum, and for this reason we suggest the name of ANAPHYLACTIN for this body.

Efforts were made to determine whether other recognizable substances of horse serum also remain in the bodies of sensitized or refractory pigs. For this purpose the sera of the animals used to transmit sensitization in Experiments 3 and 4 were tested with a potent anti-horse serum from the rabbit. One cubic centimeter of this rabbit-anti-horse serum readily gave a precipitate with .001 cubic centimeter of horse serum; the serum, however, gave no precipitate with .05, .1 or .5 cubic centimeter of sera from sensitized pig No. 33 and from refractory pigs B, C, and 4. The serum of guinea-pig No. 4, on the contrary, gave a precipitate with horse serum, showing the presence of a precipitin for horse serum.

When we consider the remarkable persistence of horse anaphylactin in the guinea-pig body, the possibility of an actual increase of this substance and not a mere resistance suggests itself. Such an increase might further be suggested by a comparison of Experiments 4 and 5 where the transmitted sensitivity would seem greater in the latter instance where the incubation period was longer. Experiments to throw light on this point were carried out in the following manner :

Experiment 6. — This experiment supplements Experiments 3 and 4, and was performed synchronously. Normal guinea-pigs were injected as follows :

G.P. No. 77. Given 1.5 cubic centimeters serum sensitized pig No. 61. (See Experiment 4.)

G.P. No. 78. Given .1 cubic centimeter serum sensitized pig No. 61.

G.P. No. 79. Given .01 cubic centimeter serum sensitized pig No. 61.

G.P. No. 82. Given 1.5 cubic centimeters serum refractory pig A.
(See Experiment 3.)

G.P. No. 84. Given .1 cubic centimeter serum refractory pig A.

G.P. No. 85. Given .01 cubic centimeter serum refractory pig A.

On injection, fifteen days later with 5 cubic centimeters horse serum, these animals reacted as follows :

No. 77. Symptoms. Chloroformed four hours. No visible lesions.

No. 78. Slight symptoms. Chloroformed four hours. No lesions.

No. 79. No symptoms. Chloroformed four hours. No lesions.

No. 82. Severe symptoms. Chloroformed three hours. Gastric hemorrhages.

No. 84. Severe symptoms. Chloroformed four hours. Gastric and pulmonary hemorrhages.

No. 85. No symptoms. Chloroformed four hours. No lesions.

This experiment would indicate quantitatively two things : First, that the blood of the refractory pig contains more anaphylactin than the blood of the sensitized pig, which is a priori evident from the much larger amount of serum received by the refractory pig ; and secondly, that the degree of transferred anaphylaxis varies with the amount of the serum used in transferring the sensitivity to a second animal.

It may further be shown that anaphylaxis cannot be transferred to a third order of animals.

Experiment 7. — Guinea-pigs No. 80 and No. 83 were treated exactly as animals 77 and 82 respectively in the last experiment, that is, they were sensitized by transfer of serum from a sensitized or a refractory pig. Normal guinea-pigs were injected as follows:

G.P. No. 111. Given 2.5 cubic centimeters serum from pig No. 80.

G.P. No. 112. Given 2.5 cubic centimeters serum from pig No. 83.

Fifteen days later these pigs were given 5 cubic centimeters of horse serum intraperitoneally. No symptoms.*

This shows that anaphylaxis, with the doses used, cannot be transmitted beyond the first transfer. This non-transference depends on too great dilution of the original anaphylactin in the animal body. It therefore seems evident that the anaphylactin of horse serum remains simply unneutralized and does not increase in amount in the guinea-pig.

Before considering the evolution of the serum disease more in detail it is important to determine if possible the identity or non-identity of the anaphylactin with the toxic element of horse serum. Such a determination presents a serious experimental difficulty. It has been shown that serum of refractory pigs No. 1 or C could sensitize normal guinea-pigs (Experiment 3); it was therefore of interest to determine if these sera would intoxicate properly sensitized pigs.

Experiment 8. — G.P. No. 55 (sensitized by injection twenty-six days previously of .01 cubic centimeter horse serum) was given 5 cubic centimeters serum of guinea-pig No. 1 intraperitoneally. No symptoms.

G.P. No. 56 (sensitized as No. 55) given 3.5 cubic centimeters serum of guinea-pig C. No symptoms.

This experiment may well be regarded as inconclusive as far as proving the non-identity of anaphylactin and toxic substance, on account of the wide divergence in doses necessary to produce anaphylaxis on the one hand and intoxication on the other. In order to demonstrate more conclusively that the anaphylactin and the toxic substance are not identical, another method of administering the toxic dose

* Another guinea-pig (No. 113), given 3.5 cubic centimeters serum from pig No. 80 and injected with five cubic centimeters horse serum intraperitoneally after thirty days also showed no symptoms.

must be mentioned. It was found that sensitized guinea-pigs are killed by very small doses of horse serum if it be given directly into the circulation. Normal pigs, however, are unaffected by a relatively large dose. The experiment follows:

Experiment 9. — 1. Normal guinea-pig, weight, 300 grams. Given 1 cubic centimeter horse serum into right carotid. No symptoms. Chloroformed 3 hours. No lesions.

2. Sensitized G.P. No. 95, weight, 340 grams. Given 1 cubic centimeter horse serum into right internal jugular. *Dead in 3 minutes.* Lungs studded with pin-point hemorrhages. Numerous hemorrhages into heart muscle.

3. Sensitized G.P. No. 93, weight, 350 grams. Given .5 cubic centimeter horse serum into right carotid. Dead in 4 minutes. One small hemorrhage on surface of brain. Numerous oozing hemorrhages of lungs which increase under observation. Smaller hemorrhages of heart.

4. Sensitized G.P. No. 98, weight, 360 grams. Given .05 cubic centimeter horse serum into right carotid. Dead in 10 minutes. Small hemorrhages of lungs. Splenic hemorrhages.

5. Sensitized G.P. No. 29, weight, 590 grams. Given .02 cubic centimeter horse serum in right carotid. Marked symptoms for an hour. Chloroformed. Gastric hemorrhages. Small lung hemorrhages.

6. Sensitized G.P. No. 35. Given .005 cubic centimeter. Very slight symptoms. Chloroformed 2 hours. No lesions.

7. Sensitized G.P. No. 97. Given 1.5 cubic centimeters serum refractory pig No. 4. (See Experiment 3.) No symptoms.

(Guinea-pigs 93, 95, 97, 98 had all been sensitized with .01 cubic centimeter 30 days previously. Nos. 29 and 35 had been sensitized with 3 cubic centimeters 60 days previously.)

This experiment brings out several novel points to which reference will later be made. It is of interest here to note that such tiny amounts of horse serum when put directly into the circulation will cause death or symptoms with lesions. This line of experimentation has been introduced for the purpose of showing that the serum of a refractory pig which contains anaphylactin does not contain any amount of the toxic substance of horse serum as far as can be detected by so delicate a biological method. We feel, therefore, justified in concluding that the anaphylactin and the toxic substances in horse serum are not identical, the one being retained in the guinea-pig body and the other

eliminated or destroyed. It would be desirable to attempt a chemical separation of these two elements.

It is already evident from the rapid evolution and often speedy termination, that this disease is due to a sharp reaction which takes place in the body, rather than a cumulative intoxication. The sharpness of the reaction is especially marked if the intracarotid method of giving the toxic dose is employed.

It may further be shown that once this reaction has taken place, the further administration of horse serum produces no effect. If the serum be administered in divided doses within a relatively short space of time, much more than a fatal dose may be given without causing death. An experiment to illustrate follows:

Experiment 10. — Guinea-pigs 94, 95, and 99 had each received .01 cubic centimeter of horse serum fifteen days previously. They were treated as follows:

No. 94, weight, 250 grams. Given 1.5 cubic centimeters horse serum *subcutaneously*.

No. 96, weight, 315 grams. Given 1.5 cubic centimeters horse serum *subcutaneously*.

No. 99, weight, 260 grams. Given 5 cubic centimeters horse serum *intraperitoneally*.

No. 99. Dead 30 minutes. Lung hemorrhages.

Nos. 94 and 96 became prostrated and were very sick at end of one hour; 94 was then given 5 cubic centimeters horse serum *intraperitoneally*, but showed no increase of symptoms and recovered in three hours; 96 received 5 cubic centimeters horse serum *intraperitoneally* 110 minutes after first injection, while still sick. No increase of symptoms. Recovered three hours.

Such experiments show conclusively that the evolution of the disease depends on some rapid reaction or assimilation. When we compare the ways of inoculation of the toxic dose, carotid, peritoneal, and subcutaneous, it is evident that the more quickly the serum comes intimately in contact with the organism the more rapid the evolution, and conversely, the smaller the dose of serum necessary to produce maximal results.

It may further be shown that mere dilution of the toxic

done with isotonic fluids tends to diminish the severity of the reaction. This dilution effect was shown by a guinea-pig which chronologically was placed in the last experiment for the sake of utilizing control pig No. 36.

Guinea-pig No. 36. Weight, 231 grams. Sensitive as No. 06, 99). Was given 10 cubic centimeters normal saline solution into the peritoneal cavity. One hour later 5 cubic centimeters horse serum was given intraperitoneally. The animal showed symptoms which were never severe and recovered perfectly in two hours.

Numerous further instances of the effect of dilution have occurred in the course of experiments carried out for other purposes. One of these may be cited.

Experiment 11.— This experiment was to demonstrate the presence or absence of anti-bodies for the toxic substance of horse serum in the serum of a rabbit which had received several injections of horse serum. (Rabbit-borne No. 1, injected 5 times with horse serum total 10.5 cubic centimeters during one month, and died 12 days after last injection. Serum heated to 56° C. for 30 minutes to destroy alexine.)

Mixtures were made of the serum of this anti-horse rabbit and horse serum, and also of the serum of a normal rabbit for control. After contact for 18 hours at room temperature the mixtures were injected into sensitized guinea-pigs. (1.01 cubic centimeter 17 days previously.)

G.P. No. 36. Weight 335. Given intraperitoneally mixture .5 cubic centimeters serum normal rabbit 56° + 5 cubic centimeters horse serum.)

G.P. No. 37. Weight 305. Given intraperitoneally mixture (.5 cubic centimeters serum anti-horse rabbit No. 1, 56° + 5 cubic centimeters horse serum).*

G.P. No. 38. Weight 345. Given intraperitoneally mixture (.2 cubic centimeters serum anti-horse rabbit + 5 cubic centimeters horse serum).*

G.P. No. 39. Weight 275. Given intraperitoneally mixture (.55 cubic centimeter serum anti-horse rabbit + 5 cubic centimeters horse serum).

G.P. No. 40. Weight 365. Given intraperitoneally 5 cubic centimeters horse serum.

Results were as follows:

No. 36. Least severe symptoms.

No. 37, 38. Severe symptoms.

No. 39. Dead in 25 minutes. Cecal hemorrhages.

No. 40. Dead in 35 minutes. No hemorrhages.

* Mixture not given until precipitate which was removed before injection.

This experiment brings out two facts. The serum of a rabbit injected several times with horse serum and bled at the critical time for antitoxins shows no anti-bodies for the toxic substance of horse serum; the non-formation of antitoxins in this experiment was confirmed both with larger doses of the serum of this specific rabbit, and also with varying doses of the serum of another anti-horse rabbit. Of importance for the present, however, is the observation that mere dilution of horse serum with the serum of a specific rabbit or of a normal rabbit decreases the symptomatic disturbance in a sensitive pig.

HISTOPATHOLOGY.

We have devoted much time to the histopathological side of the work. Despite the volume and quality of the requisite controls, we are enabled to draw a number of definite conclusions as to the structural character of the lesions found. These conclusions deal in the main with the lesions of the acute disease. We propose to return shortly in a separate paper to a consideration of some characters in the chronic or repeatedly induced disease.

The lesions of the acute disease, striking as they appear to the naked eye, yield much more to microscopic examination, since they not only are more manifold but also offer some points as to the genesis of this group of changes. We have been aided especially in the present study by the Marchi method for fatty degeneration. The crux of the work upon structural details has proved to be the investigation of the gastric mucosa, which affords a peculiarly clear field for the study of fatty changes.

Besides the hemorrhages which may be detected in the gross, there are minute interstitial and oozing *hemorrhages* which can be made out microscopically. At least a portion of these hemorrhages is demonstrably due to endothelial changes in capillaries. We are able to offer a neat histological demonstration of the fatty change which underlies this type of endotheliolysis.

Not all the lesions are capillary or hemorrhagic. *Fatty*

changes are demonstrable also in voluntary muscle fibers, in heart muscle fibers, and in nerve fibers. The hemorrhages and the above fatty changes are certainly due in some way to the experimental treatment of the guinea-pigs, and we believe them adequately controlled. The liver and the kidney also exhibit fatty changes of a similar appearance and similarly focal; but we have been unable to control the liver and kidney lesions to our satisfaction. Nevertheless, the capillary fatty change in the renal pyramids must be taken as due to the serum injections in some way. There is also a species of alteration in the large nerve cells of the spinal cord and bulb which is in our opinion produced by the treatment; but the nerve cell changes are within the limits of error in staining and are probably secondary, in any event, to the nerve fiber changes.

We have examined formaldehyde-fixed material systematically by the Marchi method⁷ for fat, from heart, lung, spleen, stomach, liver, pancreas, kidney, adrenal, cervical lymph nodes, brain, cord, peripheral nerves and muscles from fore and hind limbs, and material incidentally from several other organs. We have supplemented the Marchi method to a limited extent by fresh examination of tissues in glacial acetic acid and by staining with Scharlach R. The desire for permanent preparations and for appearances which would photograph well with high powers has led us to prefer the slower Marchi method for the bulk of the work.

Zenker-fixed tissue, prepared by the eosin and methylene blue method,⁸ has proved of service in the study of hemorrhages and alterations in heart and voluntary muscle fibers. The same series of organs and tissues prepared by the Marchi method has been carried through by the eosin and methylene blue method.

Various parts of brain and cord, with (in some instances) dorsal root ganglia, have been fixed in alcohol and stained by Nissl's original methylene blue method.⁹

The most striking feature of both the hemorrhages and the fatty changes in all the affected organs is their *focal distribution*. It is this character which demands such extensive

control and comparative work with normal and diseased tissues. This difficulty, however, is less pronounced in the case of the gastric mucosa, in which there normally exists no microscopically visible fat.

We have not been aided to any extent by accounts in the literature of experimental toxic lesions. In fact there is little evidence from published work that the histopathology in these fields is at all thoroughly wrought out. The lesions are, in the nature of things, *not specific*. It is instructive that no single finding in our work fails to find some parallel in other fields. The gastric hemorrhages themselves are found under a great variety of toxic and other conditions; it remains to be seen whether the finer anatomy in other conditions is at all constantly like that described for our own experimentally produced erosions.

We have examined material from forty-five guinea-pigs, prepared directly for this work, as well as considerable other guinea-pig material, especially diphtheria-toxon paralysis material, upon which one of us is working at the suggestion of Theobald Smith. A large number (850+) of microscopic sections accrued from the forty-five guinea-pigs alone. Each guinea-pig requires an average of eleven Marchi preparations.

We separated our material into several classes, as

I. *Control material*, consisting of supposedly normal tissues and tissues from guinea-pigs suffering from other diseases than the one under consideration and including material chiefly over and above the forty-five guinea-pigs directly prepared for the work.

II. *Anaphylaxis material*, consisting of tissues from guinea-pigs at various stages after the initial injection and before a toxic injection. Various intervals were allowed to elapse after the anaphylactic dose in different animals, so that an adequate representation could be got of conditions in the process of anaphylaxis.

III. *Toxic material*, governed in part by the frequent lethal issue, but obtained also at various intervals before death would naturally have supervened and at various intervals subsequent to recovery from toxic symptoms.

IV. *Refractory material*, composed of tissues from guinea-pigs injected with repeated large doses, obtained at intervals corresponding both with the refractory period and with a period subsequent thereto.

Stomach.—We are inclined to attach considerable significance to our findings in the gastric mucosa and vessels. Although it is probable that fatty change in the mucosa and its vessels is far more frequent than we suppose (*e.g.*, in various infections and toxic states), yet there is no reason to believe that fat is of normal occurrence in the gastric mucosa. Our own work indicates that the guinea-pig stomach under normal conditions contains no fat. The anaphylactic material also fails to show fat in the stomach wall. Many parts of the stomach in the toxic material fail to show fat. But the toxic material constantly shows fatty change of a characteristic appearance in various foci (twenty-five out of twenty-six toxic pigs examined). There is no more constant lesion anywhere in the tissues of our toxic material (Plate XII., Fig. 1). The lesion is a focal one. Intervals of tissue without fat adjoin the foci of fatty change (Plate XII., Fig. 2).

The gastric mucosa¹⁰ is a peculiarly clear field for the demonstration of fat. Very little material of any sort can be brought out by osmic acid in the gastric mucosa of guinea-pigs. The granules seen by Langley¹¹ with the employment of osmic acid, and regarded by him as related with digestion, are not alleged to be preserved in the Marchi method. Nor are these granules of Langley in the gastric epithelium, but rather in the true secreting gland cells of the gastric mucosa. The observation by Volhard¹² of a lipase in the stomach is of interest in this connection. We are not aware that the histology of the action of the gastric lipase has been worked out. Confusion with the cellular fat to which we here call attention could scarcely arise except in work on gastric absorption of fat.

Our own observations of fat in the gastric mucosa are, however, in no way equivocal or possible of confusion with

Langley's granules or with fat which might be seen in the course of absorption. Not only are our controls and anaphylactic stomachs negative for fat by the Marchi method, but also the focality of the lesion in any given stomach, together with the associated congestion and at times erosion of surface, is wholly convincing as to the pathological character of the fat shown.

The gross hemorrhagic lesion of the stomach is in our experience always associated with fat. The fat is found in two places, in the gastric epithelium (Plate XIII., Fig. 3), and in the endothelium of the neighboring vessels (Plate XIV., Fig. 6). We are inclined to regard the hemorrhages as dependent upon rupture of vessels weakened by fatty change. We are inclined to regard the fat of the gastric epithelium (Plate XIII., Fig. 4) as often independent in origin, not associated with vascular lesions. Thus we fail to find hemorrhages without fat in surrounding tissues, and we fail to find endothelial fat without fat in gastric epithelium (Plate XIII., Fig. 5); but gastric epithelial fat does occur as the unique early result of the intoxication. The gastric conditions seem to resemble logically the conditions in heart muscle, where also we find fatty changes in muscle fibers without fat in endothelial linings. In the case of heart muscle, just as in voluntary muscle, we can exclude the dependence of the fatty change in fibers upon alterations of single capillaries.

Heart muscle.— Our work with the heart muscle has concerned the question of fatty change in two tissues, the muscle fibers and the capillary walls.

We recognize that the heart muscle is not a pure field for the investigation of fatty changes. Although microscopically visible fat must be regarded as abnormal in heart muscle fibers, yet we have no adequate idea of the various non-experimental conditions under which microscopically visible fat may develop. The finding of fatty changes in the heart, as in several other organs of the so-called "normal" laboratory animal, is scarcely above suspicion in any single

response. How frequently may mild, incidental, and curable fatty changes occur in various organs, even in man? Even the present work itself tends to show how suddenly the phenomenon of microscopically demonstrable fatty change may be developed under conditions whose intimate nature we do not fully understand.

Nevertheless, our heart muscle findings are second only to the gastric findings in point of reliability. The lots of guinea-pigs employed in the present work have not been found subject to fatty degenerations of the heart muscle of non-experimental origin. Moreover, the anaphylactic material has failed in any instance to show fatty change.

We have therefore been fortunate enough to employ a variety of animals in which all the controls were free from fat in heart muscle fibers. Taken in conjunction with the focal fatty changes in capillary walls we feel that our heart findings in toxic material are almost as convincing as the gastric findings, and that they give evidence that the toxic agent takes effect (just as in the stomach) often independently in two different tissues of the same organ.

Numerically, however, heart lesions fail to equal the gastric lesions. Seven out of twenty-six pigs, examined in the toxic phase, failed to show heart lesions of any demonstrable character. An examination of the histories of the seven pigs shows that no conclusion can be drawn as to the general severity of the process from the particular incidence of lesions in the heart. One guinea-pig which showed the most manifold hemorrhages in other organs, including voluntary muscle, failed to show heart lesions of any sort. One guinea-pig, which died a little less than four hours after the second injection and showed unusually severe symptoms, failed to yield heart lesions, though the stomach wall was one mass of hemorrhages.

The maximal examples of heart muscle fiber change have been found in guinea-pigs subject to the repeatedly induced disease; to this topic we hope to return shortly in a separate paper. Nevertheless, we have found in certain pigs subject only to the two usual injections (anaphylactic and toxic) a

degree of fatty change comparable to the extensive alterations found in human fatty myocarditis (Plate XV., Fig. 9).

The capillary lesions recall those of the stomach, but are found often without associated hemorrhages or fiber changes. In fact the heart lesions afford a more convincing proof of the double action of the toxic agent (fibrolytic and endotheliolytic) than the stomach lesions. When working with the stomach lesions, we began to doubt whether the lytic phenomena could be logically separated from one another. But in the heart, as also in voluntary muscle and in the nervous system, there is the best evidence of focal changes not only in the associated tissues of a single locus but in disparate tissue elements (Plate XV., Figs. 10 and 11).

There is considerable reason to think that the hemorrhages in the heart, as perhaps in other organs, are due to ruptures of vessels consequent upon endothelial fatty change. It is surprising how difficult this is to prove. Especially in those organs which are continuously in motion, the hemorrhages speedily get beyond the range of the original focus of destruction, and their relation to endothelial changes fails of ready demonstration.

We think that our intracarotid injections with death in four minutes go far to prove the relation of the hemorrhages to endothelial change. Histological examination of the heart in such a case shows fat drops in the endothelium of many capillaries in a hemorrhagic focus.

Voluntary muscle. — It is difficult to get a numerically fair sample of voluntary muscles without carrying technical work out of manageable bounds. Confining our examination to a few muscles from the fore and hind limbs (occasionally from the back or elsewhere), we were surprised to find that fifteen out of twenty-three toxic guinea-pigs examined showed fatty degeneration in one or more muscles of greater or less degree.

The changes found correspond to those found in various febrile diseases and other conditions.¹⁸ They consist of (1) a swelling and disappearance of striations with increase of

blackness in the fiber, and more frequently of (2) the development of rows of smaller or larger fat droplets in the fiber with ultimate loss of markings. Plate XVI, Figs. 12, 13, 14.

The theoretical interest of these changes is chiefly comparative. Even more clearly than the heart fiber changes, these voluntary muscle fiber changes stand out as not secondary to vascular or local changes, but as focal cellular reactions.

Nervous system.—The same adventitious blackenings in myelin sheaths with the Marchi method have been found in our guinea-pig material as have been described so often in published accounts of work with this method.¹⁴ We have attributed no significance to these "normal" black grains, found often, as Singer and Münzer¹⁵ have described them, scattered through the spinal cord or in posterior root zones or in the arcuate fibers or raphe of the medulla. We have also met numerous examples of artificial rupture granules at the points of section of peripheral nerves.

Besides these *granular artefacts*, we find two important changes, 1, a frequent alteration found near nodes of Ranvier (here termed *nodal change*), and 2, the well-known discs or drops occupying the whole of a sheath for some distance (here termed *linear change*).

The linear degenerations are examples of the well developed and comparatively well understood Marchi degeneration, which we are accustomed to study in work on tract degenerations. Only in the present study, where we deal with effects brought about in far less time than is assigned for obtaining frank Marchi degenerations (two to four days), we discover no tract degenerations but rather isolated fiber degenerations (Plate XVII., Fig. 16). There is even a question whether it is proper to term the changes degenerations, since it is wholly probable that fibers with early linear changes may recover their myelin content without definite regeneration brought about through influences from the nervous elements engaged.

In any case, the nodal changes, which can be demonstrated as swelling and fatty transformation of the myelin sheaths on each side of the cleft of Ranvier (Plate XVII., Fig. 15), are changes of so trivial a character that they may well occur, fade, and reoccur many times without detriment to the nerve fibers.

We find focal linear alterations in our toxic material very frequently. They are the rule in the peripheral nerves, often found in entering and departing root fibers in the spinal cord and medulla, and decidedly less often in tissues above the medulla. The anaphylactic material shows very few examples of such linear degeneration. And these instances are not constant.

The nodal changes have a much wider occurrence. We find them not only in the toxic and in the anaphylactic material but also in various other material, even in some so-called "normal" material. We regard these changes as important, although not differential, in serum anaphylaxis or intoxication. They are a sign of the lability of the myelin sheath, which is here accessible to alterations by various agents.

Material from twenty-four toxic-phase guinea-pigs showed fatty alterations either in fore-leg nerves or in hind-leg nerves in nineteen subjects. Very frequently all nerves in a given subject showed the same alterations in fairly even degree throughout. Nine subjects showed the more serious changes which we have termed "linear," together with numerous examples of the "nodal" change. Ten subjects showed the nodal change alone or so few examples of linear degeneration that the findings might seem adventitious.

The question at once arose whether corresponding alterations could be demonstrated in the cells of origin for fibers so affected. Such changes might be a kind of prodrome to an actual "axonal" reaction of Nissl,¹⁶ the well-known phenomenon of granule solution and eccentricity of nucleus which occurs in nerve cells whose axis cylinders have been resected. We believe we have found such prodromata to the axonal reaction in the shape of definite reduction in size

of the Nissl bodies in certain cells which might well correspond to the fibers undergoing linear Marchi changes. Definite as these cell changes are, they are within the limit of technical error, and since they are not essential to the present discussion, we shall not go farther into the question at the present time.

To sum up our work on the neuropathological side, we have incidentally described certain myelin sheath changes which we think exemplify the remarkable lability of the myelin sheath ("nodal changes") and have made out in the anaphylactic material an accentuation of these lability changes, together with a trifling tendency to somewhat more serious and extensive changes of the myelin sheath (the well-known changes here termed "linear"). These latter changes, always focal, are displayed in greater numbers in our toxic material. These changes affect the peripheral nerves and the entering or departing root fibers in both cord and bulb. They are always focal, often not numerous, and quite resemble the voluntary muscle and heart muscle changes in focality and numerical variation.

Other organs.— We collected and examined much material from other organs.

Of these the most interesting organs are the kidney and the liver. In these organs occurred numerous focal changes, to which one might attribute significance were it not for their occurrence in "normal" and control material.

The liver shows in a small proportion of cases focal fatty alterations (Plate XIV., Fig. 7). These are, as a rule, not related with the blood supply. In a few instances the liver showed fat in capillary walls.

The kidney showed one remarkable lesion in four out of twenty-four toxic-phase guinea-pigs. The lesion failed to occur in our "normal" and anaphylactic-phase material. The lesion consisted in an exquisite deposit of small droplets of fat in the capillary walls of the pyramid region (Plate XIV., Fig. 8). This phenomenon was the more remarkable because the adjoining tubules were in most cases wholly free

from fat. The finding was in so small a proportion of cases that no extreme significance can be attached to it. It assumes logical place, however, with the capillary fat of the stomach and the heart. The peculiar locus of the change is, indeed, suggestive, because, as Dr. Councilman has remarked to us, the renal blood pressure is doubtless lowest in this region.

It is difficult to interpret findings in the lungs. Just as in the case of the heart muscle and the voluntary muscle, so also the lungs, as motile organs, so quickly disseminate a hemorrhage that the originally ruptured vessels may escape section in the embedded tissue. We have found in certain instances a small amount of fat in cells lining alveoli; but little special significance can be attached to these.

We have also paid some attention to fat findings in cells lining the pleura and pericardium. But the fat-containing cells in these loci are so few that their occurrence is of no special importance. Nevertheless, two instances of hemo-pericardium were noted among gross lesions in the toxic material.

The occurrence of fat-containing cells free in the dilated vessels of the gastric mucosa in toxic material has also a certain interest. In numerous cases the cerebral meninges showed a moderate number of fat-containing cells of the type found in many chronic lesions.

The study of the histopathology of this serum disease shows us that we have to deal with an intimate cell reaction demonstrable by definite cell lesions. We have shown that the blood of sensitized guinea-pigs regularly contains unneutralized anaphylactin, the substance in horse serum which gives rise to anaphylaxis. It would seem that the toxic substance of horse serum, so far as can be determined by biological tests, is not identical with the anaphylactin. There are two possibilities as to the cause of cellular destruction on the administration of the second dose:

First: It is conceivable that a combination between the whole horse serum and the anaphylactin of horse serum in

the guinea-pig might give rise to a toxic substance which could destroy the cells.

Second: It is conceivable that the cells have been prepared, possibly by the constant irritation of the foreign unneutralizable substance, anaphylactin, so that a sudden inrush of horse serum overwhelms them.

The first possibility may be submitted to experimental proof. In fact some experiments of Rosenau and Anderson would seem definitely to exclude this conception. These authors showed¹⁷ that mixtures of sensitive guinea-pig serum and horse serum had no toxic effect on normal guinea-pigs. It seemed to us so important to confirm this point that we repeated the experiment in a manner even more conclusive.

Experiment 12. — It has been shown that a sensitive guinea-pig can be given distinct symptoms and characteristic lesions by injecting .02 cubic centimeter of horse serum into the carotid. Normal guinea-pigs were given intracarotid the following mixtures of horse serum and the serum of a sensitive guinea-pig. The mixtures were allowed to stand 30 minutes at 37° C. before injection (no precipitate).

1. Normal guinea-pig "A," weight 325, given into right carotid (horse serum .5 cubic centimeter + serum sensitized pig 1.5 cubic centimeters). No symptoms.
2. Normal guinea-pig "B," weight 285, into right carotid (horse serum .1 cubic centimeter + serum sensitized pig 1.5 cubic centimeters). No symptoms.

This experiment proves that there is no toxic mixture formed by the interaction of the serum of sensitized guinea-pigs (anaphylactin) and horse serum.

By exclusion, we are led to assume that the intoxication in sensitive pigs is due to some change in or preparation of their cells by the sensitizing dose. An hypothesis may be offered which seems to coincide with existent facts; restating in detail our condition, the following explanation may be given of the serum anaphylaxis in guinea-pigs: There is a substance in horse serum (anaphylactin) which is not absorbed by the guinea-pig tissue, is not neutralized, and is with great slowness eliminated from the animal body; many or all of the other substances are assimilated more or less readily by the tissues, are neutralized in a relatively

short space of time, and in some instances (precipitogens) definite reaction products to them may be formed. When a normal guinea-pig is injected with a small amount of horse serum the greater part of its elements are rapidly eliminated; the anaphylactin, however, remains and acts as a constant irritant to the body cells, so that their avidity for the other assimilable elements of horse serum, which have accompanied the anaphylactin, becomes enormously increased. At the end of two weeks of constant stimulation on the part of the anaphylactin, and of constantly increasing avidity on the part of the somatic cells, a condition has arrived when the cells, if suddenly presented with a large amount of horse serum, are overwhelmed in the exercise of their increased assimilating functions and functional equilibrium is so disturbed that local or general death may occur. The period of incubation is prolonged, in the case of a large initial dose or of multiple doses, in proportion to the amount of serum injected, because the cell avidity cannot begin to increase until the assimilable substances that have been heaped up are to a large extent eliminated, and the normal assimilating function of the cell, in consequence, unsatisfied. The toxic effect of horse serum would in probability be due to such normal constituents as globulins and the great number of such elements concerned in the intoxication would perhaps preclude the possibility of neutralizing it by means of a specific serum. In the offspring of refractory mothers enough anaphylactin could pass into the fetal circulation to sensitize, but not enough of the assimilable substances to satisfy the cells. Anaphylaxis is dependent on the presence of the free anaphylactin. When the increased assimilating power of the overtrained cells of the anaphylactized animal has been satisfied, the further addition of horse serum produces no further effect. It can be conceived as possible to introduce the toxic serum, *i.e.*, the assimilable substances, so gradually as to produce no symptoms; at least, it may be said that the reaction varies directly with the size of the dose and the intimacy of its administration, or in brief, the brusqueness with which the increased cell avidity is satisfied.

As may have been deduced from our observation of the refractory guinea-pigs and their eventual sensitivity, animals which have once suffered severe symptoms and recovered, if reinjected after a sufficient period, may be made to undergo again the typical serum intoxication. By a proper dosage the disease may be repeated at relatively short intervals and as frequently as desired. By killing recovered animals at varying periods, healing lesions, such as gastric ulcers, may be studied, and, by repeating the disease, chronic conditions can probably be produced. Our observations, however, on these chronic lesions have hitherto been incidental, and we wish merely to indicate the undoubted value of a study along these lines. From the results in Experiment 9 it is evident that the localization of hemorrhages may be more or less controlled if the reaction be made sufficiently violent. In this experiment it will be noticed that the lesions correspond to the blood supply utilized and the rapidity of death; in the case of rapid death (three to ten minutes) injections into the jugular vein give hemorrhages of lungs and heart, injections into the carotid artery produce the same lesions and also affect the brain; that is, the lesser circulation alone is reached by the toxic serum; if, however, death is delayed (G. P. No. 3) gastric hemorrhages may also be produced. Injections into the left ventricle of the heart would be of interest.

In conclusion, it is of importance to consider certain general relations which exist between the anaphylactic serum and the affected organism in instances of serum anaphylaxis. It has been shown by Rosenau and Anderson that sera of animals other than the horse have an anaphylactic power against the guinea-pig. Of the sera mentioned by these authors, at least dog, cattle, and sheep are hemolytic for guinea-pig cells. Horse serum is no exception to the rule. Rosenau and Anderson through inadequacies of technic, were unable to demonstrate this hemolytic body in horse serum. Its existence, however, has been assured by competent observers and has served as the basis of an extended study by one of us¹ and may be regarded as an established fact.

It has further been shown that in processes closely allied if not identical with the instances of anaphylaxis which we have studied, there is a definite relation between hemolytic bodies and effects produced. Arthus¹⁹ found that a second injection of horse serum subcutaneously into rabbits which had previously been given horse serum at some distant point, even intravenously, gave localized necrosis; horse serum is also hemolytic for rabbit corpuscles. H. Pfeiffer²⁰ found that the necrotic action of normal sera occurs only in case the sera are hemolytic for the injected animal in question. It may be that we shall eventually find some general rule as to the concurrence of demonstrable antagonistic bodies and anaphylaxis. It may here be mentioned that the serum of the rabbit-anti-horse mentioned as of high precipitating power also contained anaphylactin, as was shown in the usual manner by injecting a normal guinea-pig and subsequently demonstrating its sensitivity (control of normal rabbit serum). This shows still further the analogy between hemolysis and anaphylaxis.

If a sensitized guinea-pig is injected with a horse serum deprived of hemolytic immune body for guinea-pig corpuscles, no decrease in the toxicity of the serum can be demonstrated. The toxic principle, then, is not dependent on the presence of the hemolytic body in this instance.

Experiment 13. — Six cubic centimeters horse serum was placed in contact with the sediment of 5 cubic centimeters of well-washed guinea-pig blood for 18 hours at 0° C. (Treated serum.)

Sensitized pigs (.1 cubic centimeter horse serum eleven days previously) were injected:

G.P. No. 3, weight, 495 grams, given 5 cubic centimeters treated serum.

G.P. No. 4, weight, 405 grams, given 5 cubic centimeters horse serum.

Both had equally severe symptoms but recovered.

The treated serum was proved by test-tube experiments to contain no longer the hemolytic immune body present in the untreated serum. It was not possible to demonstrate that the red cells of sensitized pigs are more susceptible to

hemolysis by horse serum than are the red cells of normal guinea-pigs.

CONCLUSIONS.

1. The well-known susceptibility to intoxication by horse serum, which is demonstrable in guinea-pigs previously injected with horse serum, is due to the non-neutralization and non-elimination by the animal body of a factor in the serum, for which we suggest the name ANAPHYLACTIN. The intoxication caused by the second injection depends upon factors of the serum other than anaphylactin. These factors correspond to constituents of the serum eliminable by the animal body. The reaction of intoxication would seem to be a cellular one dependent upon a heightened power of assimilation on the part of cells which have been subjected to the anaphylactic substance over a definite period of incubation.

2. The tissues of guinea-pigs examined during the anaphylactic phase show no characteristic lesions. Striking multiple hemorrhages for some reason become manifested accompany the toxic phase. The hemorrhages are more frequent in the stomach, central lungs and heart than elsewhere.

Microscopic study demonstrates that the hemorrhages are largely associated with widespread fatty degeneration of the capillary endothelium. The heart muscle, the voluntary muscle, the peripheral nerves and the gastric epithelium show striking local fatty changes which are independent of the vascular lesions.

The test of the anaphylactin is apparently so to protect various cell structures that they continued for a while in the slightly degenerated upon exposure to the toxic agent. The rapidity of this degeneration is striking though it presents morphologically the features of so-called "chronic" degeneration.

REMARKS.—As the manuscript of this article was about to be printed in the printer there came to our attention two articles, one by Besselsen and Southart² and one

by Nicolle,²³ which still further indicate the interest which attaches to this problem. Besredka and Steinhardt have added very little to the knowledge of serum anaphylaxis in the guinea-pig beyond that which had already been given by the contributions of Otto, and Rosenau and Anderson. They also have failed to note the striking lesions in this disease and have been misled into regarding the refractory condition as a state of immunity. They bring out, however, by intracerebral injections, that the intimacy with which the serum comes in contact with the body cells affects the rapidity and fatality of the disease. Our intracarotid method of injections makes this point much more evident. Their supposition that anaphylaxis depends on a sensitization of the brain is doubtless right so far as it goes, but, as we have shown, sensitivity is evidenced in the somatic cells of all parts of the body and is not confined to any definite organ.

Nicolle's contribution, while not dealing directly with the disease as we have studied it, nevertheless gives indications that anaphylactin, the unneutralized substance of horse serum, accounts for the phenomenon of localized necrosis which has been described by Arthus. It is gratifying to us that the persistence of this foreign element in the animal body seems to be a phenomenon not restricted to the single case in which we have studied it, but of a more general applicability. It is, of course, our hope that we shall be able to correlate other instances of anaphylaxis with the explanation which holds for the special instance which we have studied.

[We wish to express our thanks to Drs. W. H. Park, J. J. Kinyoun, and A. P. Hitchens for their courtesy in furnishing us with horse serum.]

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DESCRIPTION OF PLATES.

PLATE XII.

(Plates XII., XIII., and XIV., Fig. 6. *Guinea-pig stomach*.)

FIG. 1. — x 30 ca. Zenker fixation. Sharply defined focal destructive hemorrhage at base of fold in gastric mucosa.

FIG. 2. — x 45 ca. Formaldehyde fixation — Marchi. Viewed from left to right: 1. Erosion involving muscularis. 2. Fold showing epithelial fat and injected subepithelial capillaries. 3. Area of necrosis with fat. 4. Fold showing epithelial fat. 5. Deep area of necrosis with fat (obliquely set). 6. Fold showing dense deposit of epithelial fat.

PLATE XIII.

FIG. 3. — x 30 ca. Formaldehyde fixation — Marchi. Extreme injection with small hemorrhages of subepithelial capillaries. Gastric epithelium outside zone of injection shows small collections of dots, here somewhat inconspicuous, but shown with higher powers in Figs. 4 and 5.

FIG. 4. — x 1000. Below, hemorrhage from subepithelial capillary. Clusters of fat droplets in outer cells of gastric epithelium.

FIG. 5. — x 1000. Clusters of fat droplets in cells of gastric epithelium. Fat droplets lining capillary endothelium below.

PLATE XIV.

FIG. 6. — x 500. Single large subepithelial capillary distended with blood and lined with fat droplets. Occasional droplets elsewhere.

FIG. 7 (*Guinea-pig liver*). — x 30 ca. Formaldehyde fixation — Marchi. Cluster of cells containing fat. These clusters are probably not differential for the serum disease, but are found with some frequency therein. The foci are of irregular distribution and bear no special relation to the vascular supply of the liver.

FIG. 8 (*Guinea-pig kidney*). — x 1000. Capillary of pyramid region, between walls of two collecting tubules. Tubule walls free of fat. Fat droplets in capillary walls. This lesion resembles the capillary fatty change found in numerous foci of the stomach. The renal lesion is as a rule found in the pyramids where the circulation is slow.

PLATE XV. (*Guinea-pig heart*.)

FIG. 9. — x 45. Formaldehyde fixation — Marchi. Numerous sharply defined foci of fatty change. The figure represents by no means the maximum of fatty change found in some cases, but brings out the focality of the lesion. The heart, like the other organs, is not attacked *en bloc*.

FIG. 10. — x 1000. Isolated heart fiber lesion. Numerous fat droplets or drops with tendency to arrangement in rows.

FIG. 11. — x 1000. Border of hemorrhage into heart muscle. Unlaked blood. Intense fatty change in heart fibers.

PLATE XVI. (*Guinea-pig striped muscle*, formaldehyde fixation — Marchi.)

FIG. 12. — x 1000. Muscle fibers near edge of fascicle. Three types of fiber shown: 1. Normal fibers. 2. Fibers with heightened refractility. 3. Fibers with more or less numerous fat droplets, shown scattered at various intervals, because the fibers are cut in cross section.

FIGS. 13 and 14. — x 1000. Muscle fibers in longitudinal section, showing fat droplets in rows.

PLATE XVII. (*Guinea-pig peripheral nerve*, formaldehyde fixation — Marchi.)

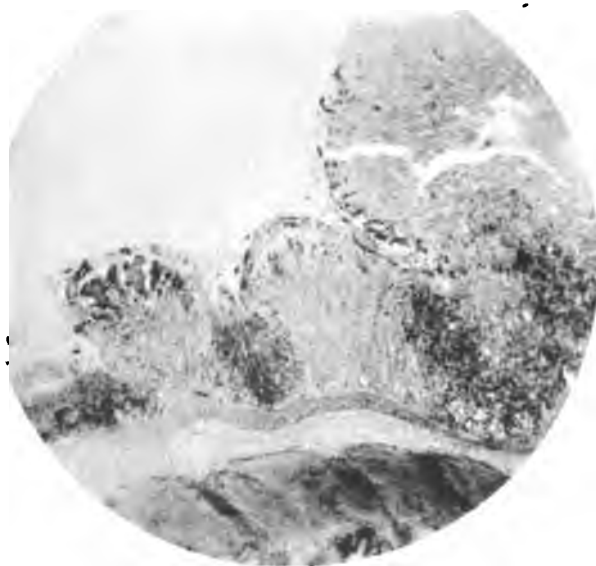
FIG. 15. — x 1000. Node of Ranvier. Swelling and blackening of myelin sheath for a brief interval on each side of the node. "Nodal change," early.

FIG. 16. — x 1000. Nodes of Ranvier. Swelling and formation of double-contoured bodies, blackened by the Marchi method, in myelin sheaths adjacent to nodes. "Nodal change."

FIG. 17. — x 1000. "Linear change." Collection of fat drops occupying site of former sheath."



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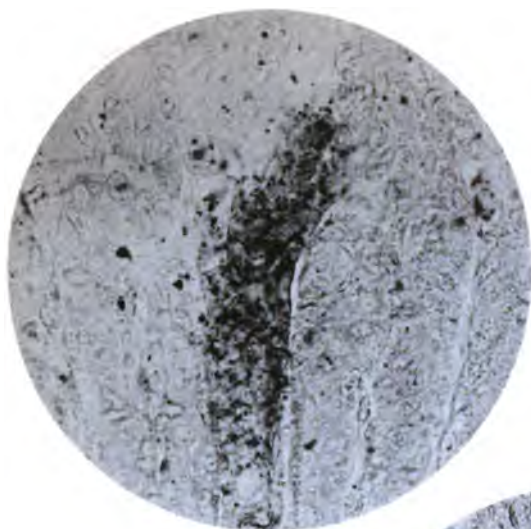
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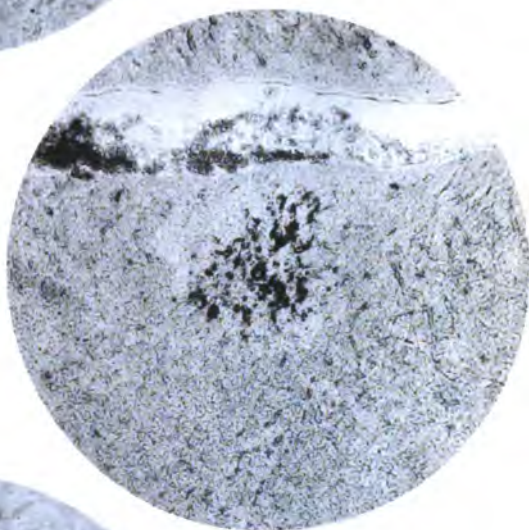
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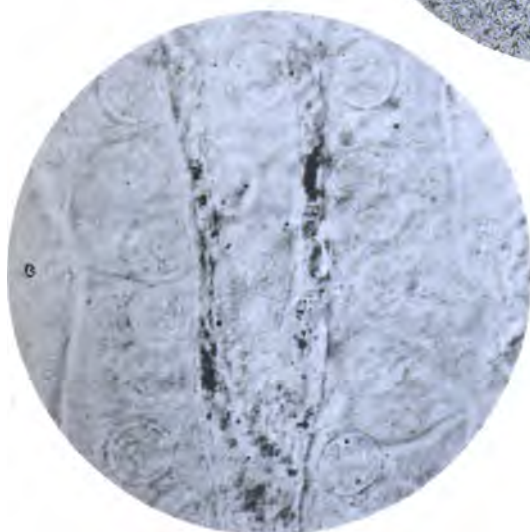
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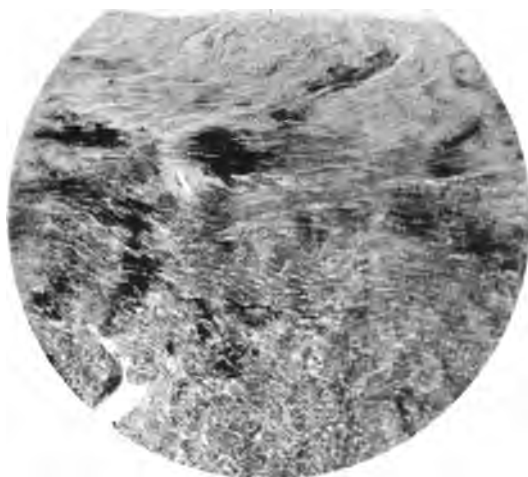
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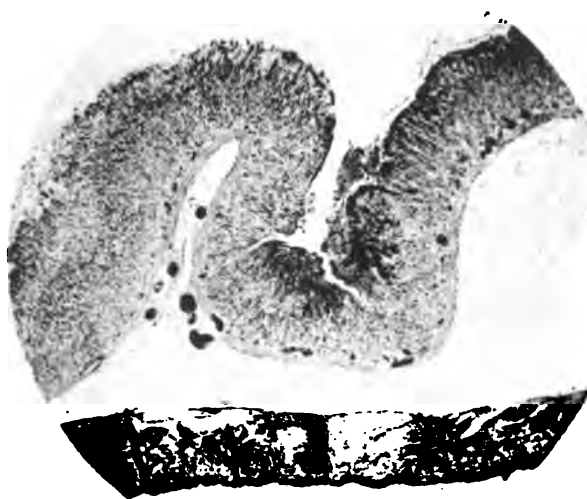
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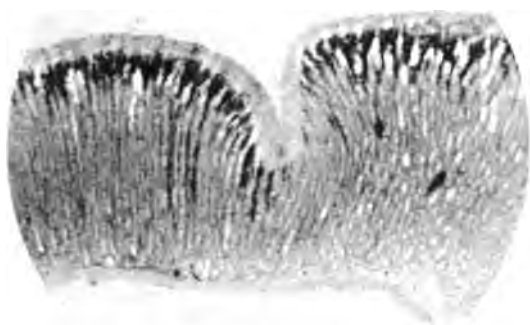
FIG. 17. — x 1000. "Linear change." Collection of fat drops occupying site of former sheath."



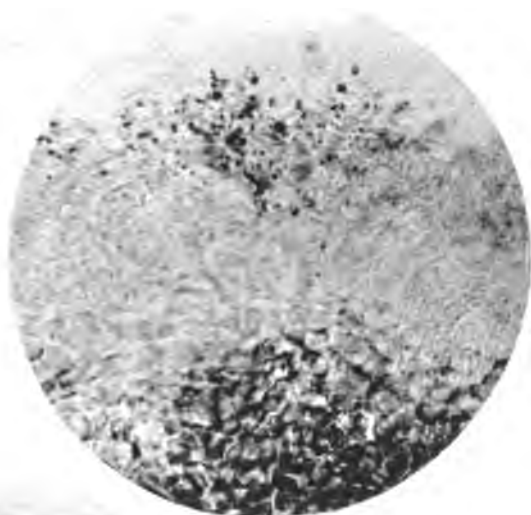
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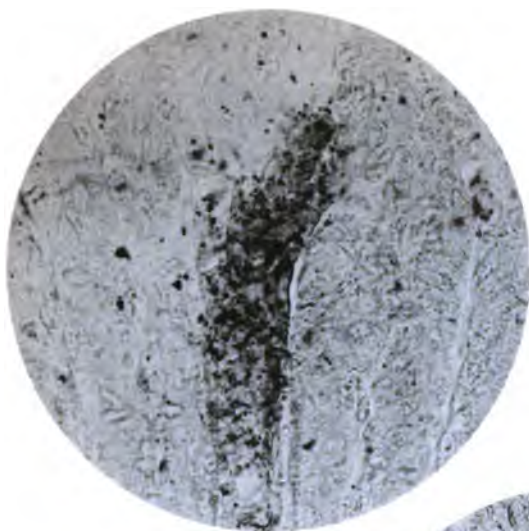
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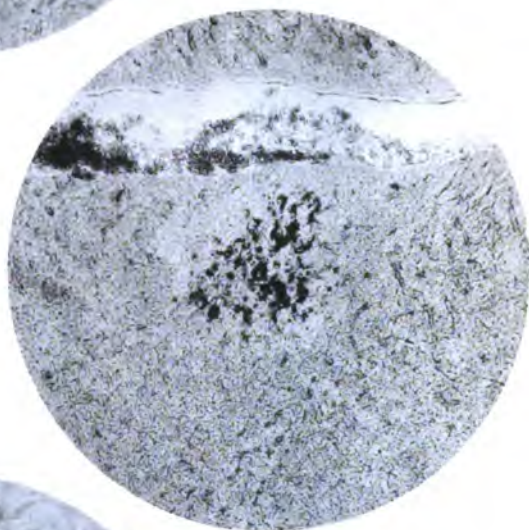
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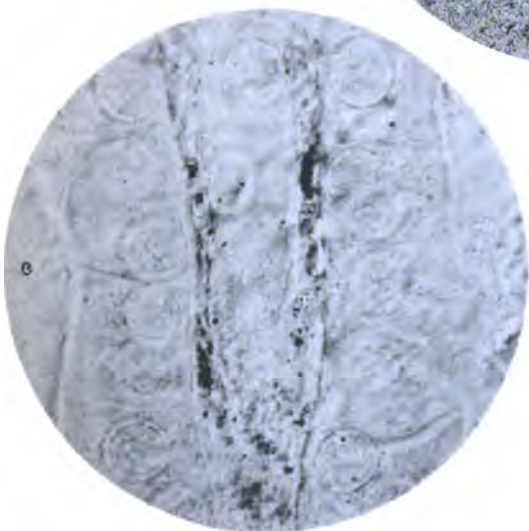
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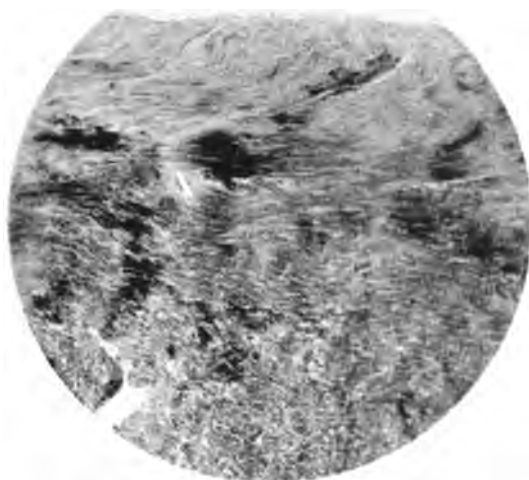
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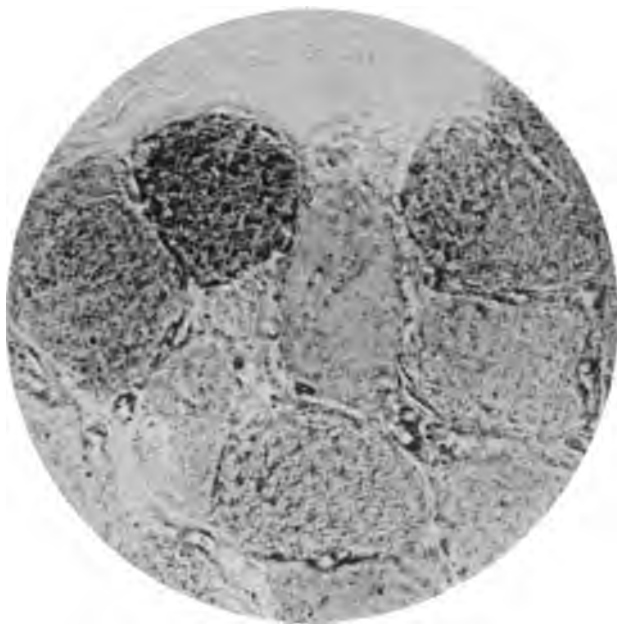
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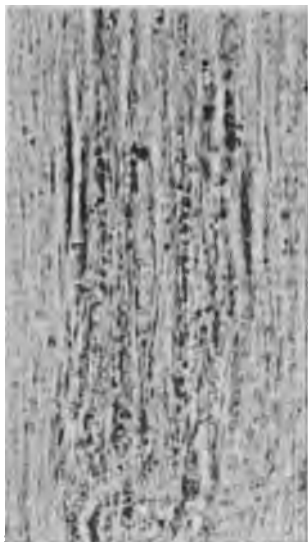
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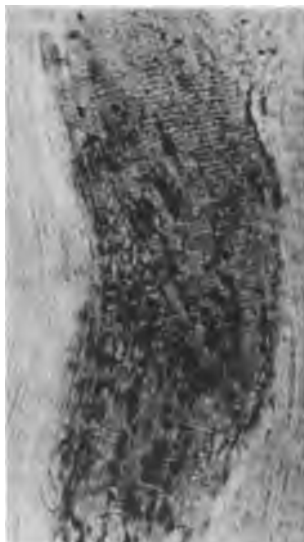
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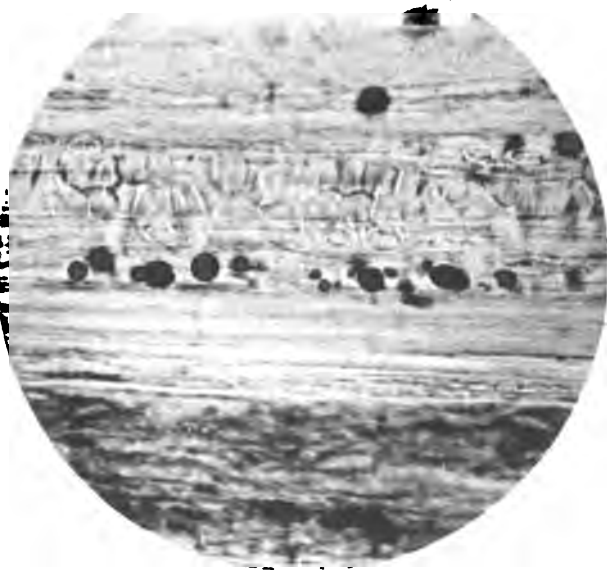
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A STUDY OF NORMAL AND DIARRHEAL STOOLS FOR THE
DETECTION OF DYSENTERY OR ALLIED ORGANISMS,
WITH DESCRIPTION OF A NEW BACILLUS.*

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(*From the Laboratory of the Connecticut Hospital for the Insane.*)

In an effort to ascertain the mode of infection in asylum dysenteries, Dr. Simon Flexner suggested the study of normal stools as well as mild cases of diarrhea with a view to determining the presence or absence of the dysentery or allied organisms.

The report of this investigation naturally divides itself into a brief review of the literature of the subject, a general discussion of the methods of obtaining specimens from normal cases, bacteriological technic, a summary of two cases with a description of a new organism, its morphology, cultural features, and agglutination reactions.

Recently there has been much work done upon and considerable discussion of dysentery organisms varying in minor characteristics from the original type and from each other, but all undoubtedly belonging to the dysentery group. There are other organisms, however, which have not established their claim to this distinction, notably the organisms producing atypical reactions in milk. It was one of these latter which we isolated from normal stools.

In 1897, Shiga found a bacillus in dysenteric stools which has since been looked upon as the true dysentery organism. In 1900, Flexner¹ isolated a bacillus from dysentery which he at first considered identical with that found by Shiga in Japan. Later it was discovered to differ in many particulars. Kruse in Germany isolated a similar bacillus.

Strong² also recovered a bacillus, which later was found to differ from Shiga in its reaction on mannite as well as in its agglutination reaction. Park and Dunham isolated another organism in 1902, which differed in agglutination reaction and indol production. Duval³ described another organism which he considered a member of the dysentery group. This organism differed from the other types of dysentery bacilli in its action on lactose and litmus milk and its agglutination reaction which had a striking resemblance to typhoid.

*Received for publication Feb. 15, 1907.

Latterly the subdivision into types has been much studied on a basis of the action on carbohydrates and the agglutination reaction. On the former basis Hiss⁴ proposed the division into four groups.

The first group consists of the Shiga type, fermenting monosaccharids. The second group splits monosaccharids, mannite, saccharose and maltose (bacillus Y of Hiss). The third group differs very slightly from group two, principally in agglutination reaction. It is more active on saccharose. This difference, however, does not seem sufficient to warrant a separate grouping. The fourth group is represented by the Flexner type and approaches more nearly the type of the bacillus coli communis. It ferments the monosaccharids, mannite, dextrin, and saccharose. If the lactose fermenting organisms (Duval and Shorer) are to be considered as members of the dysentery group there will need to be a still further subdivision.

Torrey⁵ would include all mannite fermenters in the dysentery group provided the reaction on litmus milk was typical. He considers this cultural test the most reliable. The organisms differing in their reaction on litmus milk he would class as pseudo-dysentery bacilli.

Previous investigations⁶ from this laboratory have established the fact that asylum dysentery is identical with the dysentery occurring outside of insane hospitals and is undoubtedly due to *Bacillus dysentericus* (usually the mannite fermenters), and not to any inherent influence of the asylum, its environment, or insanity itself, as has been supposed.

Thirty-seven patients were studied in this investigation; eighteen normal and nineteen suffering from mild diarrhea. The stools used in examination were fresh, being received at the laboratory always within one-half hour after evacuation. Many plates were made from each case and all colonies bearing the faintest resemblance to the dysentery organism were picked off. The number picked sometimes ran up into the thousands, which was in accord with the purpose to study exhaustively a few cases rather than to make a superficial examination of a larger number of cases.

The normal cases were carefully selected; none but individuals in perfect physical condition and free from previous attacks of dysentery were accepted for the experiment and most of these patients were inclined to constipation. It was a question how best to obtain a suitable specimen from a healthy individual without disturbing the

balance of health in the intestines. A perfectly normal stool afforded a poor outlook, because from fecal material used for plating there was such a wealth of colonies that it seemed a hopeless task to isolate an organism which might be expected to be present in very small numbers, if present at all, under normal conditions.

At the suggestion of Dr. W. H. Park the fenestrated rectal tube (a small blind glass tube with a small lateral opening) and swab was employed in six cases as follows: after an enema of normal salt solution was given, the flushing being completed, a sterile, blind fenestrated tube was carefully inserted into the rectum a distance of four or five inches, the mucus swabbed from the rectal wall and inoculated immediately in sterile bouillon. This method has the advantage of absolute freedom from outside contamination, also perfect freshness of the material and a healthy condition of the rectum, hence the material used was as near normal as it was possible to obtain. This procedure, however, has disadvantages, in that the material is from the rectum alone instead of from a more extensive intestinal surface, it is disagreeable to the patient, and required time from the laboratory. Furthermore, it did not give as good results as the cautious use of cathartics.

Three cases were taken from post-mortem scrapings from the colon of patients, one of whom died from mural thrombosis of the heart and chronic nephritis, one from apoplexy, and the other from phthisis. Three specimens were taken from stools secured for biochemical tests after special diets and were absolutely normal except for a little mucus coating in one and a little bloody mucus in the other, the latter probably due to hemorrhoids. In these cases the mucus was used in plating. The other six subjects were given a cathartic, usually a saline. The fourth or fifth stool was selected for plating, as this was found to contain the most mucus and a minimum of fecal material. While the mucus of these stools was undoubtedly produced by the irritation of the cathartic, it does not seem probable that the condition of the intestine was so altered as to affect in any way the bacterial flora.

The use of the fenestrated tube yielded a "dysentery-like organism" only twice out of six cases ($33\frac{1}{3}$ per cent). From the action of cathartics this organism was isolated four times out of nine ($44\frac{4}{9}$ per cent), while scrapings from the colon post-mortem gave the highest number, two out of three cases ($66\frac{2}{3}$ per cent), which would seem to indicate that the habitat of this organism was more often the colon than the rectum.

The Hiss plating media⁷ was used and from thirty to fifty plates made from each stool. The dilution found to give the most uniform and useful plates was one small loop of a bouillon suspension of mucus to a large tube of medium (about 30 cubic centimeters) making two or three plates each. Plates made in this manner produced from one hundred to three or four hundred colonies each.

Plates were never discarded until they were too dry for further growth, and from many plates every colony was picked. This wholesale stripping of plates was done to remove all doubt as to the possibility of overlooking the dysentery organism which one would naturally expect to find in limited numbers, if present at all, in the normal bowel. The colonies were transplanted to the Hiss semisolid medium,⁸ which was found invaluable in eliminating gas producing or motile organisms. The non-motile and non-gas producing cultures were transferred to litmus milk and slant agar, while more minute differentiations were made by means of mannite agar and litmus serum water media (Hiss). All litmus milk tests as well as the fermentation of sugars in litmus serum water were made several times and observations taken every day for thirty days unless, as occasionally happened, the media dried during the last few days of observation.

In ten of the normal cases studied, no dysentery or "dysentery-like organism" was isolated. In eight, two of which were autopsy cases, many colonies of a "dysentery-like organism" (pathogenic to guinea-pigs) were recovered. For convenience this organism will be designated *Bacillus F*. In the second autopsy case an organism (pathogenic to

guinea-pigs) resembling *Bacillus fecalis alcaligenes* was isolated. It differed from the alkaline organism of Duval, in that it did not split either dextrose or saccharose and produced but a faint reaction in mannite. It was pathogenic to guinea-pigs and was recovered in large numbers from one normal case.

Fifteen diarrhea cases were negative, that is no dysentery or "dysentery-like organisms" were recovered. From two cases *Bacillus F* was isolated the same as from the eight normal cases. One case of severe diarrhea gave very many colonies, in fact almost a pure culture of a small diplococcus which died out too soon for thorough study. The colonies were very fine, dew-like, and did not appear on plates under thirty-six or forty-eight hours.

Two out of the nineteen cases of diarrhea yielded the dysentery bacillus of the Flexner type (one in small and the other in large numbers). Of these two cases, the first subject was a man fifty-nine years of age who worked every day on the lawn. The previous summer he had a severe attack of diarrhea, but no examination was made of the stools. The attack under consideration began May 10, 1905. On the first day he had two soft movements. On the second there were eighteen stools which were scanty, dark brown liquid, containing flakes of mucus with feces and shreds of undigested food. On the third day under treatment, the stools were reduced to three and were semisolid and slate color. On the fourth, fifth, and sixth days the bowels did not move and a cathartic had to be administered. His temperature was 101° F. on the second day, but gradually dropped to normal. The stool used in examination was passed the second day and contained little flakes of mucus, liquid feces and undigested food, but no blood. The mucus was used in plating. Forty-seven plates were made and nine hundred and fifty-one colonies were picked, all of which proved to be gas producers on semisolid media except four, which were among the last thirty colonies tested. This shows the value of testing many colonies and the futility of superficial examinations in work of this character.

These four organisms were non-Gram, non-motile bacilli giving an acid, amphoteric then alkaline reaction on milk and fermented mannite agar without the production of gas. They fermented saccharose, dextrose, mannite, galactose, maltose, and glucose, but did not change dextrin, inulin or lactose. They were agglutinated in dilutions up to one thousand with Flexner (horse) serum and were positive with the Shiga (horse) serum and his own serum at one to two hundred.

Blood taken seven days after the onset, when the patient had practically recovered, gave positive agglutinations with the paracolon bacillus (1-40), colon bacillus (1-20), but was negative with Shiga, Flexner, "Mt. Desert," and "Coney Island" dysentery bacilli.

This patient did not resume regular diet but continued to take milk, eggs, and toast, when, ten days after the beginning of the first diarrhea, he had a severe attack of dysentery lasting about ten days (from which recovery was at one time doubtful). The stool examined from this attack contained much blood and little mucus but no feces. Eighteen plates were made, from which one hundred and eighty-one colonies were picked, when three colonies of the Flexner type of dysentery organism were isolated and the search was discontinued. The identification of these organisms was complete. The agglutination with Flexner (horse) serum was 1-500, with Shiga, 1-200, with his own serum, 1-50.

The second case of diarrhea from which the Flexner type of organism was obtained occurred in a woman forty-nine years of age. The attack lasted two days, during which time she had numerous watery mucus stools. The first day the temperature was 100.6° F., gradually dropping to normal. During the second day of the disease a specimen was sent to the laboratory. It was liquid, containing feces in suspension, also vegetable fibers and had a peculiar strong odor. Neither blood nor mucus being present, it was necessary to use the flakes or feces in making the twenty-five plates. One hundred and sixty cultures were the result of the first day's picking, about fifty of which were non-gas producers on semisolid media (Hiss). Of these fifty, only twenty-four

were further tested and identified with the Flexner type of organism. The serum from this patient agglutinated the Flexner organism in dilution of 1-100, paracolon 1-100, colon 1-50, and own organism 1-250, and the bacillus was agglutinated by Flexner serum (horse) 1-1,000.

The organisms from both of these cases were pathogenic to guinea-pigs in the same dose as the Flexner bacillus. It was impracticable to use feeding tests so intraperitoneal inoculations were resorted to. The animals sickened at once, lying in their cages, refusing food and dying in from twelve to twenty-four hours. At autopsy, the abdominal cavity contained a little bloody serous exudate and the liver was covered with little flakes of fibrinous material. The peritoneum was slightly hyperemic.

In one case of dysentery a bacillus resembling Duval's (*Bacillus A*) was recovered in large numbers and *Bacillus F* in small numbers. This case, an old woman ninety-three years of age, suffered from a simple diarrhea beginning July 6, 1905. July 7th, forty-three plates were made from a rectal swab. No mucus was obtained and the plates were negative. There were but few suspicious colonies and only four hundred and forty-five were picked. Eight days later the patient passed blood and mucus, which was received at the laboratory on a sheet. Thirty-five plates were made from this bloody mucus and four hundred and forty-six colonies were picked, thirty-six of which were first acid on litmus milk, then amphoteric, finally permanently acid without coagulation until the media dried up. These organisms fermented maltose, galactose, mannite, dextrose, and glucose, but not saccharose, lactose, inulin or dextrin. They were agglutinated by the Harris (horse) serum in dilutions of 1-1,000, but were negative to Shiga serum. A few cultures of *Bacillus F* were also isolated.

One of the negative cases of diarrhea was periodic in character and an examination was made from a rectal swab in the interval between attacks as well as stools from two different attacks. The two diarrheal cases from which *Bacillus F* was isolated differed in no respect from others from which it was not obtained.

DESCRIPTION OF BACILLUS F.

Morphology. — The morphological character of this bacillus is of no value for its ultimate classification, because it is not distinguishable from bacilli of the colon and dysentery groups. The bacillus varies considerably, like the dysentery and colon, in different media varying with the temperature, etc., but it is generally a short rod with rounding ends from .3 to .6 of a micron in width and from one to four microns in length. Fresh broth cultures are usually very short rods, while older cultures are much longer. It sometimes occurs in pairs but is usually single. No spores, capsules, or flagella were discovered. It stains readily with the usual stains and is decolorized by Gram's method.

Biology. — It is an aërobic, facultative anaërobic, non-liquefying bacillus, developing best at 37° C., but grows very well at room temperature. It is non-motile except for a slight Brownian movement, occasionally observed. It develops well on all the usual culture media, but the differences in growth are not sufficient to differentiate it from *Bacillus dysentericus*. On gelatin plates the colonies were not distinguishable from the dysentery colony and there was no liquefaction, although the colonies had a very moist appearance. On the gelatin stab cultures there was no liquefaction, and the growth was identical with that of the Flexner bacillus. On the Hiss plating media the surface colonies, about the size of dysentery colonies, are slightly wavy in outline, grayish translucent in color by transmitted light, few are umbonate, all are slightly elevated and moist in appearance. Microscopically, they are uniformly granular with central thickening, edges transparent and occasionally scalloped. The deep colonies are round or slightly lenticular in shape and creamy in color. On agar slants the growth is a grayish translucent streak with slightly wavy edges spreading out in bulb shape at the bottom. In bouillon they give a uniform turbidity without the formation of a pellicle. On potato, the growth is creamy in color unless it is slightly seeded, when it is almost invisible. Litmus milk is changed

to a lilac red color, a brighter tint than that produced by the dysentery bacillus, in the first twenty-four hours, and continues so without coagulation until the medium dries up, or for five or six weeks. This medium is the most satisfactory early differentiator between this bacillus and the members of the true dysentery group. On mannite litmus agar it produced acid without gas formation, and the appearance of the growth was not distinguishable from the Flexner type of *Bacillus dysentericus*. On litmus serum sugars (Hiss) *Bacillus F* ferments lactose, maltose, saccharose, galactose, dextrose, mannite, and glucose, but gives no appreciable reaction on dextrin and inulin.

Litmus serum media in the differentiation of colon, Shiga, Flexner, Bacillus F, and the Duval organisms.

List of Sugars.	Colon. (With Gas.)	Bacillus F. (No Gas.)	Flexner. (No Gas.)	Shiga. (No Gas.)	A. B. C. (Duval and Shorer.)
Lactose	++	++	—	—	+++
Maltose	++	++	++	—	+++
Saccharose.....	++	++	+	—	— + +
Dextrin.....	++	—	+	—	— + +
Galactose	++	++	++	++	
Dextrose	++	++	++	++	+++
Inulin (decolorized) ...	++	—	—	—	
Mannite	++	++	++	—	+++
Glucose	++	++	++	++	

The litmus serum tests were made three times and in some cases four, extending over a period of at least thirty days with daily observations by the same individual. The variation in these three different tests was so great that we have come to consider this medium an unreliable differential test. The medium was prepared with extreme care and the sugars used were from the same package, yet occasionally a given organism would give a different reaction in each of the three

tests as —, +, + —, but more often two tests would be identical, in which case the predominant reaction was taken as the true one. Whether this variation was due to faulty technic, unavoidable variation in medium, to oxidation or other chemical change, or to variations in amount or activity of bacteria inoculated, it would be impossible to say. It would seem, however, that if a medium prepared with extreme care had to be inoculated and reinoculated before the true reaction could be determined, that it is entirely too unstable and requires too much time to be of any practical value in the differentiation of bacteria.

Bacillus F did not produce indol after repeated tests, but the presence or absence of indol in cultures of the dysentery type is of no value for differential purposes. It was pathogenic to laboratory animals in the same doses as the Flexner type of dysentery, and the same conditions were found post-mortem after intraperitoneal injections; namely, bloody serous exudate, hyperemia of peritoneum and intestines and a fibrinous exudate on the liver. One rabbit, which died during immunization, had little yellowish nodules scattered through the liver. Microscopically, these were encysted necrotic areas without bacterial invasion.

Bacillus F inhibits the growth of the Shiga and the Flexner type of organisms in broth cultures and on agar plates. The bacteria were cultivated separately in neutral bouillon for twenty-four hours without any alteration in the reaction of the medium. At the end of that time the cultures were mixed and allowed to stand for another twenty-four hours at room temperature before plating. The Hiss plating medium was used and the plates yielded a pure culture of Bacillus F showing that the bacteria were in some way antagonistic to each other, and that Bacillus F was the surviving organism. This enantiobiosis may account for the fact that the bacteria were found but once associated together in any of the one hundred and thirty-five dysentery cases previously examined here, also for the absence of the bacillus dysentericus from normal stools.

Agglutination. — The serum reaction has its limitations as a differentiator because of a lack of absolute specificity, but at the same time it has its value when carefully used. Experience with the agglutination reaction tends to show that wide variations in agglutinability may occur in different strains of the same organism and that in estimating the agglutinable value of a serum or organism both values must be considered. Buxton and Vaughan⁹ have clearly demonstrated this in their work with different strains of typhoid bacilli.

In this connection many agglutination and absorption tests were made with rabbit immune serum. The work was undertaken primarily to show the variation in agglutinability of some of the types of dysentery organisms in comparison with *Bacillus F*. Later this developed into a study of the comparative agglutinability of various species of bacilli when inoculated simultaneously, a report of which is given in the following article, "A Study of Agglutination."

The organisms used in these experiments were the Shiga bacillus obtained through Dr. Flexner; Flexner bacillus (Dr. Flexner); Colon bacillus (Dr. W. H. Park); Typhoid bacillus (Yale Pathological Laboratory); Paratyphoid bacillus (Dr. Herter's Laboratory); and *Bacillus F* isolated by us from normal intestines.

Rabbits were used in the immunization experiments. They were all healthy animals and none were utilized if their normal serum agglutinated any of the immunizing organisms in a dilution of 1-10. They were immunized by means of bouillon cultures sterilized in the autoclave for fifteen minutes at 60° C., under five pounds pressure. Inoculations were made for three successive days, then once a week for two or three months. The initial dose was one cubic centimeter gradually increased to eight to ten cubic centimeters. For the simultaneous inoculations the cultures were mixed just before the administration of the dose. Two animals succumbed during the process of immunization apparently from toxemia. They were the ones inoculated respectively with

Flexner and Bacillus F necessitating another immunization. The other animals showed only a temporary reaction to even the largest doses of combined bacilli.

Testing of agglutinations.—There has been a wide variation in the technique of different workers in agglutination with a consequent disparity in results. Many observers prefer the macroscopic method, at the same time admitting that they control all doubtful cases if not every case, by means of the microscope. Macroscopic tests undoubtedly facilitate the work, but the microscopic tests are much finer, more reliable and less subject to variation. For this reason, and because the amount of serum available was limited, the microscopic test has been used exclusively in this work. Formalized salt solution emulsions adopted by Buxton and Vaughan¹¹ did not work as satisfactorily in our hands for microscopic as for macroscopic experiments, hence the fresh bacillus cultures were adopted in these experiments. A control test was invariably made, thus obviating possible mistakes from pseudo-clumps. The readings were made at the end of two hours.

There was a wide variation in the agglutinability of the six organisms used in these experiments, the typhoid bacillus heading the list in every combination of sera. Bacillus F generally came next in agglutinable value followed closely by the Flexner and colon bacilli. The Shiga bacillus was usually agglutinated at a much lower dilution, while the paratyphoid culture was the poorest agglutinator of all. Once paratyphoid in combination-serum reached a positive reaction at 1-1,000, but with its own serum alone it never agglutinated above 1-500.

The symbols used in the following tables are ++ (double plus) indicating a reaction in which every bacillus was clumped, the clumps being large; + (plus) indicates a reaction where the organisms were all agglutinated, but the clumps were smaller; +- (plus and minus) stands for a partial agglutination with some free organisms, and - (minus) indicates no reaction.

TABLE I.
Agglutination of Bacillus F by rabbit immune sera.

[illegible]

It will be remarked in the first table, as would be expected, that *Bacillus F* was agglutinated at the highest dilution by its own serum. Gradually, however, the agglutinable value of the serum diminished as other organisms were combined with *Bacillus F* for immunization. Why the combination of organisms should diminish the agglutinable value of the serum is not plain, unless the presence of an increased number of common agglutinins interfered with the reaction, which would be contrary to the observations of Buxton and Vaughan. The common agglutinins are generally supposed to supplement the action of the specific agglutinins, thus increasing the reaction.

The combination serum *Bacillus F* and typhoid agglutinated *Bacillus F* at a much higher dilution than did the serum from the combination of *Bacillus F* and Flexner, while the typhoid serum alone had no agglutinating power, and Flexner serum alone was + in dilution 1-100. The same thing will be observed in regard to *Bacillus F* and paratyphoid serum which agglutinates ++ 1-5,000, + to 10,000. The addition of the paratyphoid organism in immunization cut down the agglutination from 1-40,000 (*Bacillus F* serum) to 1-10,000 in combination, while the paratyphoid serum alone gave no reaction with *Bacillus F*.

Bacillus F serum agglutinated *Bacillus F* at 1-50,000; Lindberg 4 (a Flexner type isolated from a case of diarrhea previously described) was agglutinated at 1-5,000; Parker 1 (a Duval type) at 1-1,000, while the original Flexner organism (Harris) reached only up to 1-100 and Shiga at 1-40.

After absorption with *Bacillus F* the agglutinable value of this serum was reduced for *Bacillus F* to 1-500, and for Flexner all agglutinins were removed.

TABLE III.
Bacillus F serum absorbed with *Bacillus F*.

Culture.	Before Absorption.	After Absorption.
<i>Bacillus F</i>	50,000	500
Lindberg 4 (Flexner)	5,000	500
Parker 1 (Duval type)	1,000	20
Flexner (Harris)	100	0
Shiga	40	20

From this absorption it would seem that there was a closer relation between *Bacillus F* and Duval's type than between *Bacillus F* and the dysentery bacillus, although our Lindberg 4 culture, which in other respects is identical with the Flexner culture, shows a much higher agglutination and greater absorption. Our Duval type, however, produced no specific agglutinins for *Bacillus F*, reacting in dilutions of 1-20 only, while the typhoid serum induced no reaction (+ 1-20), and the colon serum very little.

When the absorption of *Bacillus F* serum was made with the Flexner organism, the agglutinable value of the serum for *Bacillus F* was reduced to thirty thousand, and was negative for Flexner.

TABLE IV.
Bacillus F serum absorbed with Flexner (Harris).

Culture.	Before Absorption.	After Absorption.
Bacillus F	50,000	30,000
Lindberg 4 (Flexner type).....	5,000	5,000
Parker 1 (Duval type).....	1,000	40
Flexner (Harris)	100	0
Shiga	40	20

The Flexner organism removes only the common agglutinins from Bacillus F serum.

When Flexner and Bacillus F were both used in the immunization the agglutination of both organisms was reduced. Bacillus F with own serum agglutinated 1-50,000; with combination Flexner and Bacillus F serum only 1-4,000; Flexner with own serum 1-40,000; with the combination 1-4,000. This serum after absorption with Flexner was negative with Flexner bacilli and agglutinated Bacillus F only in 1-40 dilution, when absorbed with Bacillus F the condition was reversed. This would seem to show that there were more common than specific agglutinins present in the serum or that the antagonism noticed between the two organisms in vitro held good in vivo. In all sera there was a noticeable decrease in the agglutinating power of the combination-serum with every organism added to the combination, but this was most noticeable where Bacillus F was one of the immunizing organisms. The typhoid bacillus was least affected by combinations, and was always agglutinated higher than any other organisms of the combination.

TABLE V.
Flexner (horse) serum.

With this serum it will be noticed that *Bacillus F* is agglutinated at a lower dilution than the colon, and the agglutination is undoubtedly due to the common rather than the specific agglutinins.

TABLE VI
Flexner horse serum absorbed by Bacillus F.

Culture.	Before Absorption.	After Absorption.
<i>Flexner</i>	50,000	4,000
<i>Bacillus F</i>	200	0
Shiga	1,000	100

Bacillus F has removed many of the agglutinins for the Flexner organism, and for Shiga as well as for *Bacillus F*.

TABLE VII
Flexner 'horse' serum absorbed by Flexner bacilli.

Culture.	Before Absorption.	After Absorption.
<i>Flexner</i>	50,000	100
<i>Bacillus F</i>	200	40
Shiga	1,000	200

The Flexner organism has not absorbed as many agglutinins for Shiga from this serum as did *Bacillus F*.

The agglutinability of the bacillus must always be considered as well as the agglutinable value of the serum, but in these experiments the bacillus was always the same except for the slight variation due to renewed cultures. The group or common agglutinins in these cases have not supplemented the specific ones in their action causing an increased agglutinating strength, as would be expected, but have undoubtedly retarded or weakened the specific agglutinins themselves.

SUMMARY,

All cases, however brief the duration, where blood or bloody mucus was found in the stool, invariably yielded the bacillus dysentericus of the Flexner type, with the exception of two mild cases, in one of which the Shiga type was recovered and in the other the Duval lactose fermenting type.

The Flexner type of *Bacillus dysentericus* was recovered twice in simple diarrheas, once in very small numbers (four colonies out of about one thousand picked), the second time in large numbers.

The Flexner organism may occasionally be a factor in the production of mild diarrheas, as it was recovered in two out of nineteen cases after prolonged search.

The Shiga type was never found in simple diarrheal stools or in normal intestines.

No type of the bacillus dysentericus was isolated from normal stools.

Bacillus F, a "dysentery-like" organism, was recovered from 44.4 per cent of normal stools, from 10.5 per cent of simple diarrheas and from .01 per cent of dysentery cases.

In agglutination and absorption experiments *Bacillus F* produces specific agglutinins for *Bacillus F*, but not for types of dysentery, colon or typhoid, and differs from all three organisms in its reaction on litmus milk and with some of the carbohydrates.

The frequent presence of *Bacillus F* in the intestines of the healthy individual renders it of interest and importance because of the close resemblance to the bacillus dysentericus.

Bacillus F was isolated from one case of dysentery and two of simple diarrhea, and its close resemblances in morphology, cultural characteristics, agglutination and absorption reactions place it undoubtedly in the classification with the organisms described by Duval and Shorer and termed by Torrey pseudo-dysenteries.

This organism would seem to be much more closely related to the pseudo-dysentery group (Torrey) than to either the dysentery, colon, or typhoid groups.

Bacillus F differs from Duval's organisms in the production of permanent acidity on litmus milk without any amphoteric stage, in its pathogenicity to guinea-pigs and negative reaction with typhoid serum.

It differs from *Bacillus A* (Duval) in that it ferments saccharose; from *Bacillus B* (Duval) in that it does not ferment dextrin but does ferment saccharose; and from *Bacillus C* by not fermenting dextrin.

Bacillus F differs from the colon bacillus in that it does not coagulate milk, is not motile, is not agglutinated by the colon immune serum, nor does *Bacillus F* serum agglutinate the colon bacilli. The colon bacillus ferments all sugars with the production of gas, while *Bacillus F* does not form gas with any of the carbohydrates and does not ferment dextrin or inulin.

Bacillus F inhibits the growth of both the Shiga and Flexner type of dysentery organism in vitro.

Torrey suggests the possibility that these lactose fermenters may by a gradual change of habitat and evolution lose their power to ferment carbohydrates and thus in time approach the Shiga type. This suggestion hardly seems tenable as cultures under experimentation for a considerable time have not changed perceptibly in their characteristics.

[The thanks and acknowledgments of the writer are due to Dr. Simon Flexner and Dr. A. R. Diefendorf for their advice and assistance in this work; also to Dr. A. B. Coleburn for cases and material.]

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A STUDY OF AGGLUTINATION.*

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The object of this investigation has been a study of the variation in agglutinability of the different types of dysentery organisms in comparison with *Bacillus F*,¹ together with the comparative agglutinability of various other species when simultaneously inoculated. This includes observations upon rabbit sera, human normal sera, and human dysenteric sera.

The value of the agglutination and absorption reactions as a means of differentiating various types of dysentery organisms has been the subject of inquiry because of the lack of absolute specificity of the agglutinins produced by intestinal bacilli. These organisms give rise to many common as well as specific agglutinins which influence every reaction. The agglutinable value of bacteria fluctuates widely, even varying in the same cultures from changes of temperature, age, etc. The number of organisms in an emulsion must also be considered, for, the greater the number of bacteria to be agglutinated, the greater the number of agglutinins which will be necessary to produce a complete reaction. Many factors, therefore, must be considered in interpreting agglutination reactions, notably the agglutinable value of the serum as tested upon a known type of bacilli, the agglutinable value of the organism as tested by a known serum, the presence or absence of agglutinoids, and the number of organisms present, as well as the technic of the operation.

METHODS. — In these experiments the microscopic method was used exclusively in the agglutination reactions because we found it could be read in higher dilutions than the macroscopic. For example: in two experiments made in this laboratory with typhoid bacilli and typhoid serum, we

* Received for publication April 1, 1907.

found a positive reaction at 1-40,000 in the hanging drop while the test-tube was negative at 1-10,000.

Eighteen to twenty-four-hour bouillon cultures made from recent agar slants were used for the agglutination reactions and a control test was always made, thus obviating possible mistakes from pseudo-clumps.

Healthy rabbits were used in the immunization experiments, and none were utilized if their normal serum agglutinated any of the intestinal organisms used in this work at 1-10. This is an important consideration, as sometimes there are present in the blood of both animals and man agglutinins for intestinal organisms. This occurred twice out of thirty rabbits. Once we found that the Flexner organisms were agglutinated at 1-200, and once the typhoid bacilli at 1-2,000 by the serum of apparently healthy rabbits.

The six organisms used in the immunization were:

The Shiga bacillus obtained through the courtesy of Dr. Simon Flexner (Rockefeller Institute).

The Flexner bacillus also obtained from Dr. Flexner.

The colon bacillus obtained from Dr. W. H. Park (Board of Health Laboratory, N.Y.).

The typhoid bacillus obtained from Prof. C. J. Bartlett (Yale Laboratory).

The paratyphoid bacillus obtained from Dr. C. A. Herter's Private Laboratory, N.Y.

Bacillus F isolated from normal intestines at the Laboratory of the Conn. Hospital for the Insane.

Intraperitoneal inoculations for immunizations were made on three successive days, then once a week for three months. The initial dose administered was one cubic centimeter of a twenty-four to forty-eight hour bouillon culture sterilized in the autoclave for fifteen minutes at 60° C. under five pounds pressure. This dose was slowly increased until the animals took without discomfort eight to ten cubic centimeters of the bouillon culture.

For the simultaneous inoculations of more than one organism, the cultures were mixed after sterilization and just before the administration of the dose. The total quantity of

the dose in the simultaneous inoculations naturally had to remain the same as that of the separate organisms, hence the quantity of each organism entering into the combination was reduced in proportion to the number of organisms entering into the combination; for instance, the initial dose was one cubic centimeter, hence if one organism was used there was one cubic centimeter of that bacillus administered, if two organisms one-half cubic centimeter of each was given, if three organisms one-third cubic centimeter of each, and so on.

The blood serum of the animal was tested every week or ten days until it reached the height of its agglutinability for the organisms of immunization; that is, when the reaction remained practically stationary for two weeks. The rabbit was then bled from the carotid artery with aseptic precautions, and the serum, without the addition of any preservative, sealed in sterile bottles and placed on ice.

The agglutinating power of this serum was tested within a few days of its preparation with nineteen stock organisms, again after absorption with each organism used in immunization, and finally after absorption with all the organisms used in immunization. Of these nineteen organisms, we will omit from consideration, for the sake of brevity, five organisms of the Flexner type, one of the Duval lactose fermenting type, two alkaline organisms, and two of the Bacillus F type (all isolated in this laboratory) and confine this report to our Bacillus F and eight of the well-known types of intestinal organisms, namely the Shiga, Flexner, paratyphoid, typhoid, Coney Island, colon, paracolon and Mt. Desert bacilli.

RABBIT IMMUNE SERA. — It scarcely seems necessary to tabulate all of the agglutination and absorption experiments on rabbits, therefore this report will be limited to examples showing:

- (a.) Variation in agglutinability before and after absorption.
- (b.) The lack of specificity of agglutinins.
- (c.) Reduction of agglutinating strength of immune sera by the use of more than one organism in immunization.

- (d.) The supplementing of specific agglutinins by common agglutinins.
- (e.) Variation in the agglutinable value of the different organisms.

Theoretically, simultaneous injections ought to produce specific agglutinins for each organism which could be removed by absorption without affecting the agglutination of other organisms. In this connection, we may refer to Buxton and Vaughan,² who have given very convincing examples to prove this. In our work this theoretical result has not been demonstrated.

(a.) Variation in agglutinability before and after absorption.—In some cases absorption with one organism has materially decreased the agglutination of others and almost invariably affected it to some degree; for example, in the combination serum, induced by inoculation of *Bacillus F* and the typhoid bacillus, typhoid bacilli were agglutinated at 1-500,000 and *Bacillus F* at 1-30,000, but after absorption with the typhoid bacilli, the serum still agglutinated the typhoid bacilli at 1-50,000, but was entirely negative with *Bacillus F*, but when absorbed with *Bacillus F* the typhoid reaction was reduced to 1-10,000, a greater reduction than after absorption with typhoid bacilli.

TABLE I.
Immune rabbit serum Bacillus F and typhoid.

Organisms.	Before Absorption.	After Absorption with Typhoid.	After Absorption with <i>Bacillus F</i> .
<i>Bacillus F</i>	1-30,000	0	0
Typhoid	1-500,000	1-50,000	1-10,000

In a few instances, absorption by one of the immunizing organisms has seemed to free receptors for one of the other immunizing bacilli, which agglutinated higher after absorption than before. Examples: In the Shiga and

typhoid serum before absorption the typhoid bacilli were agglutinated 1-5,000, but after absorption with Shiga the typhoid agglutination went up to 1-100,000, and, after absorption with the typhoid bacilli, instead of decreasing the original reaction it increased it to 1-20,000. The Shiga agglutinins apparently inhibited the action of the typhoid agglutinins and their removal increased the reaction.

TABLE II.
Immune rabbit serum Shiga and typhoid.

Organisms.	Before Absorption.	After Absorption with Typhoid.	After Absorption with Shiga.
Shiga	1-1,000	1-500	0
Typhoid	1-5,000	1-20,000	1-100,000

Shiga and paratyphoid serum agglutinated the Shiga bacilli 1-500 and the paratyphoid bacilli 1-1,000 before absorption, but after absorption with paratyphoid bacilli, Shiga was agglutinated ++ 1-3,000 and + 1-5,000.

TABLE III.
Immune rabbit serum Shiga and paratyphoid.

Organisms.	Before Absorption.	After Absorption with Shiga.	After Absorption with Paratyphoid.
Shiga	1-500	1-1,000	1-5,000
Paratyphoid	1-1,000	1-500	0
Flexner.....	1-1,000	1-100	0
Typhoid	1-500	1-2,000	0
Coney Island	1-500	0	1-100
Colon	1-20	0	0
Bacillus F.....	1-20	0	0
Paracolon	1-100	0	0
Mt. Desert.....	1-20	1-20	0

Here again it is observed that there is a higher agglutination of Shiga and typhoid after absorption by Shiga, one of the immunizing organisms.

(*b.*) Lack of specificity of agglutinins. — In the Shiga and paratyphoid serum agglutinins were produced for the Flexner, typhoid, and Coney Island bacilli at as high a dilution as for the bacilli of immunization, but for the other organisms to only a slight degree. These agglutinins were removed for all except Coney Island by the absorption with the paratyphoid bacilli, showing that the reaction was due to common agglutinins.

From the agglutination reactions shown in Table III., before absorption it would be impossible to differentiate the organisms used in immunization because of the height of the reaction of the others, due undoubtedly to the large number of common agglutinins.

(*c.*) Reduction of agglutinating strength of immune sera by the use of more than one organism in immunization. — Bacillus F and the Flexner bacilli used together in immunizing gave rise to agglutinins for both organisms, but neither organism was agglutinated in as high dilutions as when a single organism was used in immunization. For instance, Bacillus F immune serum agglutinated Bacillus F in a dilution of 1-50,000, while the combination immune serum of Bacillus F and Flexner agglutinated Bacillus F in dilution of 1-1,000 and Flexner 1-3,000. The Flexner serum alone agglutinated the Flexner bacilli in a dilution of 1-10,000. This was true in varying degrees of all double combinations of Bacillus F except in the case of typhoid and Bacillus F, in which both organisms were agglutinated as high as in the separate immune sera.

TABLE IV.
Table showing the highest agglutination of the different organisms when acted upon by single or combined immune rabbit serum.

Serum.	Colon.	Typhoid.	Paratyphoid.	Bacillus F.	Flexner.	Shiga.
Each serum acted upon by its own immune serum.....	+ + 4,000 + 5,000	+ + 100,000	+ + 40 + 500	+ + 50,000	+ + 10,000	+ 2,000
Combination serum Bacillus F and typhoid.....	** + + 500,000	+ + 4,000 + 30,000
Bacillus F and paratyphoid.....	+ + 100 + 500	+ + 500 + 10,000
Bacillus F and Flexner.....	+ + 40 + 1,000	+ + 200 + 3,000
Bacillus F and colon.....	+ + 500 + 2,000	+ + 2,000 + 5,000
* B serum.....	+ 10,000	+ + 100,000	+ 100
* C serum.....	+ 1,000	+ + 40,000 + 50,000	+ 100	+ + 2,000 + 4,000
* K serum.....	+ 1,000	+ 4,000	+ 1,000	+ 500
* E serum.....	+ 100	+ + 100,000	+ 40	+ 4,000	+ + 1,000	+ 100
* J serum.....	+ 4,000	+ 3,000	+ + 1,000
* H serum.....	+ + 4,000	+ 40	+ 2,000	+ + 2,000	+ 3,000
* D serum.....	+ 2,000	+ + 100,000 + 30,000	+ 100	+ 2,000	+ 1,000

** Only time tested in such high dilution, so cannot be compared. * B serum: Colon, typhoid, and paratyphoid. * C serum: Colon, Flexner, typhoid, and paratyphoid. * K serum: Bacillus F, colon, Flexner, and Shiga. * E serum: Bacillus F, colon, Flexner, Shiga, typhoid, and paratyphoid. * J serum: Colon, Flexner, and Shiga. * H serum: Bacillus F, colon, Flexner, Shiga, and paratyphoid. * D serum: Colon, Flexner, Shiga, typhoid, and paratyphoid.

The typhoid bacilli are the strongest of all organisms in both their agglutinogenic power and agglutinating value, and are but little affected in multiple immune serum. The paratyphoid bacilli, the poorest agglutinators, had the least agglutinogenic power and the typhoid bacilli the greatest. The colon and Shiga bacilli once showed an increase in reaction, otherwise the agglutinating strength of multiple immune sera was less than serum acted upon by a single organism. The reduction in the amount of the dose administered in the simultaneous inoculations probably did not affect the number of agglutinins produced because some organisms; namely, typhoid, Shiga, and colon were observed several times in higher reactions in multiple than in single sera.

(d.) The supplementing of specific by common agglutinins. — The results of absorption tests were not constant, varying fully as much as did the agglutination reactions. Sometimes absorption by an organism would not remove all agglutinins for itself from a multiple serum, showing that the specific agglutinins were supplemented by the common agglutinins raised by the associated bacilli. A few examples are given below.

TABLE V.
Immune rabbit serum Flexner and typhoid.

Organisms.	Before Absorption.	After Absorption with Flexner.	After Absorption with Typhoid.
Shiga	20	0	0
Flexner	40,000	100	50,000
Paratyphoid	100	0	0
Typhoid	100,000	5,000	40,000
Colon	500	0	1,000
Bacillus F	40	0	40
Paracolon	3,000	500	5,000
Mt. Desert	500	0	40
Coney Island	1,000	0	5,000

In this experiment the absorption by typhoid bacilli did not remove as many agglutinins for the typhoid bacilli as did the absorption by the Flexner bacilli. Absorption with typhoid bacilli raised the reaction for the colon, paracolon, and Coney Island bacilli.

TABLE VI.
Immune rabbit serum colon and typhoid.

Organisms.	Before Absorption.	After Absorption with Typhoid.	After Absorption with Colon.
Shiga	20	0	0
Flexner.....	100	0	20
Paratyphoid	40	0	0
Typhoid	100,000	500	30,000
Colon	3,000	500	100
Bacillus F	500	40	40
Paracolon.....	1,000	40	200
Mt. Desert.....	40	0	0
Coney Island.....	40	0	0

Neither colon bacilli nor typhoid bacilli removed all agglutinins for themselves, but absorption by each materially decreased the reaction of the other organisms. Paracolon, as frequently happened, reacted quite high, although it was not used in any of the immunizations.

TABLE VII.
Immune rabbit serum typhoid and paratyphoid.

Organisms.	Before Absorption.	After Absorption with Paratyphoid.	After Absorption with Typhoid.
Shiga	20	20	0
Flexner	100	20	20
Paratyphoid	200	0	40
Typhoid	50,000	5,000	5,000
Colon	20	0	0
Bacillus F.	100	20	20
Paracolon	1,000	100	40
Mt. Desert	40	20	0
Coney Island	100	0	0

Absorption by typhoid bacilli did not remove all agglutinins for the typhoid bacilli any more than did the paratyphoid absorption. The paracolon bacilli were again agglutinated higher than the paratyphoid bacilli, which was one of the immunizing organisms.

TABLE VIII.
Immune rabbit serum (B): Colon, paratyphoid, and typhoid.

Organisms.	Before Absorption.	After Absorption with Colon.	After Absorption with Typhoid.	After Absorption with Paratyphoid.
Shiga	20	20	0	0
Flexner	200	0	100	40
Paratyphoid	100	0	20	0
Typhoid	100,000	3,000	3,000	10,000
Colon	10,000	1,000	5,000	3,000
Bacillus F.	20	0	0	0
Paracolon	200	1,000	1,000	5,000
Mt. Desert	20	0	0	0
Coney Island	0	0	0	0

The colon absorption did not remove all agglutinins for the colon bacilli, but reduced the typhoid reaction as much as did the absorption by the typhoid bacilli.

Absorption by the paratyphoid bacilli reduced the reaction of both the typhoid and colon bacilli, but increased the reaction of the paracolon bacilli, which was originally higher than paratyphoid, one of the immunizing organisms.

TABLE IX.
Immune rabbit serum Bacillus F and paratyphoid.

Organisms.	Before Absorption.	After Absorption with Paratyphoid.	After Absorption with Bacillus F.
Shiga	20	0	0
Flexner	100	20	0
Paratyphoid	500	0	40
Typhoid	200	20	0
Colon	0	0	0
Bacillus F.	10,000	4,000	1,000
Paracolon	200	40	100
Mt. Desert	0	0	0
Coney Island	0	0	0

Absorption with Bacillus F failed to remove all agglutinins for Bacillus F, but reduced the paratyphoid reaction to 1-40. Absorption by the paratyphoid removed all agglutinins for that organism and decreased the reaction of Bacillus F from 1-10,000 to 1-4,000.

On the other hand, an organism sometimes removed all agglutinins for both immunizing organisms, which would seem to show that no specific agglutinins had been raised by the other organism or else that the specific agglutinins were the same (which is highly improbable) for the two organisms. For example:

TABLE X.
Immune rabbit serum Flexner and paratyphoid.

Organisms.	Before Absorption.	After Absorption with Flexner.	After Absorption with Paratyphoid.
Shiga	20	0	0
Flexner.....	20,000	0	10,000
Paratyphoid	100	0	0
Typhoid	20	20	0
Colon	40	0	0
Bacillus F.....	200	20	200
Paracolon	20	20	0
Mt. Desert	100	0	200
Coney Island	100	0	1,000

The Flexner organism removed agglutinins for both organisms of immunization.

Absorbed with the paratyphoid bacilli the Flexner reaction was reduced one-half, but the Coney Island reaction was much increased.

Bacillus F reacted in a higher dilution than did the paratyphoid bacillus, which was one of the immunizing organisms.

The absorptive power of organisms other than those used in immunization was tested twice, once with the Flexner bacilli acting upon the serum of Bacillus F and once with Bacillus F acting upon the Flexner (horse) serum. Practically the same result was obtained in each case; namely, a slight reduction in the reaction for the inoculating bacillus and an entire removal of all agglutinins for the absorbing bacillus, showing that other organisms do not affect the specific agglutinins but remove only those common to both organisms. In but one instance (serum colon, Flexner, and Shiga) were all agglutinins removed by absorption with all the organisms of immunization.

TABLE XI.
Immune rabbit serum Flexner, colon, and Shiga.

	Before Absorption.	After Absorption with Flexner.	After Absorption with Colon.	After Absorption with Shiga.	After Ab- sorption with all the Organisms of Immuni- zation.
Shiga	1,000	200	100	0	0
Flexner.....	3,000	0	40	500	0
Paratyphoid	0	0	0	0	0
Typhoid	100	0	0	100	0
Colon	4,000	2,000	0	4,000	0
Bacillus F	40	20	0	40	0
Paracolon	0	0	0	0	0
Mt. Desert.....	40	0	0	40	0
Coney Island.....	40	0	0	20	0

This is the only serum from which all agglutinins were removed by absorption with all the organisms of immunization.

The reaction of both Shiga and Colon was considerably reduced after absorption by the Flexner bacilli, showing that the specific were supplemented by the common agglutinins.

(e.) Variations in agglutinable value of organisms.— There was considerable variation in the reaction of each organism with the different combination sera of which it was one of the immunizing bacteria. Colon showed the lowest agglutination reaction when acted upon by the combination immune serum of colon, typhoid, paracolon, Bacillus F, Flexner, and Shiga. The typhoid reaction was lowest with the multiple immune serum from colon, typhoid, paratyphoid, and Flexner. The paratyphoid reaction was never high and did not vary as much as the others, although it showed the same tendencies. Bacillus F agglutination was lowest (1-1,000) with multiple serum Flexner and Bacillus

F. Flexner was lowest with multiple serum colon, Bacillus F, Flexner, and Shiga. Shiga, next to paratyphoid, was the poorest agglutinator. It reacted lowest with the combination serum from all the organisms. (See E serum, Table IV.)

In these experiments it was found that the agglutinating strength of a given serum may vary from time to time; for example, Bacillus F immune serum agglutinated Bacillus F + + 1-20,000 and + 1-40,000 when drawn, while three months later the same serum, kept on ice without the addition of any preservative, agglutinated Bacillus F + + 1-50,000, which was 30,000 higher than when the serum was first drawn and tested. This serum did not deteriorate on standing, neither were the agglutinins changed to agglutinoids.

Human normal serum. — The serum from four normal individuals (laboratory staff) showed the presence of agglutinins in all but one case for one or more of the following organisms: Flexner, Mt. Desert, Coney Island, and paracol. In the first case the Flexner organisms were agglutinated at 1-20. In the second case the Mt. Desert and Coney Island bacilli were agglutinated at 1-500. The typhoid, Shiga, and colon bacilli were negative with sera from all four individuals. This demonstrated that mistakes may easily be made in the use of the serum reaction in diagnosis when the normal reaction of the individual is unknown.

Human dysenteric serum. — It is of interest to note the results of four cases of agglutination and absorption of human serum of patients from whose dysenteric stools the bacillus dysentericus (Flexner type) was isolated. The blood for these absorptions was drawn by means of a ten-cubic-centimeter glass hypodermic syringe, with antiseptic precautions, from the median or medianbasilic veins. The patients suffered no inconvenience from the loss of such a small quantity of blood and the only local manifestation was a slight ecchymosis due to the leakage from the needle puncture. The sera from these four subjects contained

agglutinins in dilutions varying from 1-250 to 1-1,000 for the Flexner type of organism isolated from their own stools. It also possessed agglutinins in practically the same dilutions for the original Flexner, Mt. Desert, and Coney Island bacilli, as well as for the colon and paracolon bacilli. The Shiga bacilli did not react higher than 1-20.

Absorption tests made in the usual way with the dysentery organism isolated from the individual in two of the above cases removed all agglutinins from the serum, showing that the infection was a single one, that the organism isolated was the infecting agent, and that the agglutinins affecting the other organisms were common agglutinins induced by the same organism. From the third case all agglutinins were removed except those for the paracolon bacilli, the reaction of which was reduced from 1-1,000 to 1-50. In the serum of the fourth case the agglutinins remained after absorption for both the colon and paracolon bacilli in practically the same dilution as before absorption, showing that in all probability this individual's blood contained agglutinins for these two organisms before the onset of the attack of dysentery. This experiment shows that it is impossible by serum diagnosis to absolutely differentiate the type of the infecting organism in dysentery. These cases are of more value than animal experiments because there can be no question of agglutinins induced artificially.

SUMMARY.

The use of more than one organism in animal immunization tends to reduce the agglutinins produced for each of the inoculating bacilli. This might possibly be explained in two ways: first, by the over-stimulation of the cell due to the action of so many organisms, causing it to give off fewer specific agglutinins; secondly, by considering that a given cell is capable of producing only a certain number of agglutinins. When this cell is acted upon by a single organism all of these agglutinins would be excited, but when two or more organisms are used in immunization, the number of agglutinins being limited must be divided

between these organisms, resulting in a decrease in the agglutination of each.

The simultaneous inoculation of various types of bacilli produces specific agglutinins for each organism, but not in as great numbers as when each organism is separately injected.

The agglutination reaction has a very limited value in the differentiation of types of intestinal organisms.

In these experiments almost without exception, absorption of a multiple immune serum by one of the immunizing organisms lowered (in many cases materially) the agglutination reaction of the other immunizing organisms.

Absorption of a multiple immune serum by one of the organisms of immunization did not always remove all agglutinins for that organism, showing that the reaction was supplemented by the common agglutinins raised by the other organisms.

The agglutinogenic power varied with the agglutinable value of the organism.

Normal human and animal serum may contain agglutinins for one or more organisms in dilutions as high as 1-2,000, therefore the reaction of a serum must be ascertained before beginning animal experiments or interpreting clinical agglutinations.

In the human or animal subject, the agglutination reaction is of no value in differentiating the type of the infecting dysentery bacilli.

[The thanks of the writer are due to Dr. Simon Flexner, who suggested this problem, and to Dr. A. R. Diefendorf for his hearty coöperation and critical assistance in this work, also to Mr. Victor Caryl Myers for valuable help in immunization, etc.]

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THE APPLICATION OF THE CARD-CABINET SYSTEM IN THE STORAGE OF MICROSCOPICAL SLIDE-PREPARATIONS.*

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To those engaged in histological study, the method to be employed in the storage of slides is a matter for consideration. The problem of storage is perhaps most difficult in the pathological laboratories connected with the large hospitals, where it is necessary to file away the slides of a large number of cases, or in departments of instruction in which a great many sets of histological preparations are kept for teaching purposes. Even the investigator may be at a loss how to dispose of his steadily accumulating slides. Every one dislikes to throw away preparations which may be of use to others or which may be valuable for future reference, yet unless some efficient method of storage is employed, they not only occupy more space than is necessary, but are often difficult to find when most desired. A system of storage should be simple, compact, and of the easiest possible access.

Perhaps the most serviceable of the slide cabinets heretofore devised are those with trays in which the slides lie horizontal. Cabinets of this type are especially useful in storing new preparations, since they must be kept in the horizontal position sufficiently long after mounting for the balsam to harden. However, for the storage of older preparations these cabinets are not only bulky but expensive.

No one ever undertakes to store cards in a horizontal position in trays, and there is no valid reason why old and thoroughly dried slides should be stored in this manner. The method here proposed is simply an application of the card-cabinet idea to the storage of such slide-preparations as are to be kept for future reference or for teaching purposes. A

* Received for publication March 8, 1907.

cabinet of the following design has been given a thorough trial and has proved efficient in every respect. This has



twelve double trays, each of which is divided by a median longitudinal partition so as to accommodate two rows of slides stored on edge. The trays have depth sufficient to allow for cardboard markers in addition to the width of the slides, are about twelve and three-fourths inches long, and seven inches wide. They should be well made of thoroughly seasoned wood, preferably of hard wood, and finished within and without. The frame for holding the trays is constructed after the manner of card-cabinets in general. Especial attention should be given to the matter of strength in its construction, for when filled with slides the weight represented is considerable. All the joints of the frame, as well as of the trays, should be dovetailed where possible and elsewhere neatly joined. Such a cabinet having twelve double trays occupies a space approximately $16 \times 13 \times 13$ inches and holds six thousand slides of medium thickness. This

furnishes a unit of convenient size, as one of greater dimensions would be of too great weight to be readily moved about. A cost of \$19.00 is an estimate given, by one of the manufacturers of card-cabinets, for the construction of a single cabinet of this description. At this figure the cost per slide for storage is less than one-third of one cent.

The form may be modified to meet the requirements of the case. Single instead of double trays may be preferred by some, although the latter are not too heavy to be handed about with ease. Others may prefer to store the slides on end, which necessitates deep trays. Such a system has already been devised, but the difficulty in manipulating the slides in this position furnishes an objection. Shallow drawers may be divided by partitions at appropriate intervals and made to hold large numbers of slides, but are not so convenient as trays of such size that they can be carried about without effort.

The limitations of the system of storage proposed are obvious, but I am nevertheless convinced that it will be found a most useful as well as simple method of storing large numbers of histological slide-preparations. It cannot be used for fresh preparations, as these must be kept for a time in the horizontal position. Immersion oil should be removed from the slides before they are stored in the manner proposed, although even this is not disastrous, for preparations which are adherent are readily pulled apart and cleaned. On the other hand, by this method a large number of slides may be kept together in small space and in such a manner that they are always accessible and ready for reference.



THE ESTIMATION OF LEUCOCYTES FROM STAINED BLOOD-SMEARS.*

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This paper represents a study of the value and accuracy of certain attempts to simplify the process of blood-counting by substituting the use of stained smears for the Thoma-Zeiss apparatus.

Such a method was used by Einhorn in 1884. It was further elaborated by Einhorn and Laporte in 1902 and subsequently by Hewes. The two last methods are almost identical. Smears are made and stained in the ordinary manner. The leucocytes in a certain number of fields are then counted, parallel counts being made with the Thoma-Zeiss pipettes and counting-chamber. This is done in a series of cases, the larger the better. One is thus able to determine the arithmetical relationship between the number of leucocytes in a definite area of the smear and the actual number in a cubic millimeter. In subsequent cases the number per cubic millimeter may be found by multiplying the number in the same area of the smear by the constant factor thus obtained. The arithmetical methods vary in detail but are alike in principle.

As the area of the microscopical field varies with the instrument used, each investigator must establish his own standards by a series of parallel smear-counts and Thoma-Zeiss counts; or he may, within certain limits, adjust his field to correspond with that of a previous investigator by varying the lenses and tube-length. The ruled lines on the Thoma-Zeiss slide afford a convenient micrometer for this purpose.

As to the accuracy of these methods, Einhorn and Laporte were able, in three to five minutes, to make white counts whose errors, compared with the Thoma-Zeiss method,

* Received for publication April 1, 1907.

TABLE I.
The asterisk (*) indicates errors which would have had clinical importance.

Number of Case.	Diagnosis.	Inspection.		Smear-counts.				Thoma-Zeiss.				Hemoglobin, per cent.
		Leucocytes per cu. mm.	Per Cent Error.	Leucocytes per cu. mm.	Time in Minutes.	Per Cent Error.	Whites.		Reds.			
							Per cu. mm.	Time in Minutes.	Per cu. mm.	Time in Minutes.		
1....	Influenza.	13,000	+53	6,829	11	-20	8,500	13	6,804,000	35	95	
2....	Chronic acetanilide poisoning.	6,000	-6	4,500	8½	-30	6,400	14	3,168,000	25	70	
3....	Acute rheumatism.	12,000	+13	21,495	13	*+103	10,600	15	4,589,300	27	93	
4....	Splenic anemia.	6,000	*-72	17,072	12	-21	21,600	13	2,595,000	22½	70	
5....	Anemic child.	5,000	-20	7,838	9	+26	6,200	13	4,152,000	20	76	
6....	Splenectomy.	12,000	+11	11,795	7	+9	10,800	10	4,584,000	27	95	
7....	Empyema.	8,000	*-51	11,097	8	*-32	16,300	10½	2,608,000	12	35	
8....	Chlorosis.	5,000	-43	7,294	9	-18	8,800	19	3,464,000	27	36	
9....	Chlorosis.	5,000	-43	9,467	9½	+8	8,800	15	4,008,000	26	52	

10....	Addison's disease	4,000	-31	5,742	7	-1	5,800	13	5,272,000	33	70
11....	Chlorosis	10,000	+9	10,088	84	+10	9,133	20	4,640,000	32	95
12....	Rickets	12,000	-22	28,557	10	*+85	15,400	13	3,640,000	25	60
13....	Secondary anemia	16,000	+1	17,150	10	+9	15,800	124	4,888,000	27	55
14....	Debility	12,000	-4	12,338	74	-1	12,500	12	4,352,000	27	107
15....	Chronic arthritis	4,000	-47	6,130	64	-19	7,600	15	5,333,000	26	115
16....	Fatal anemia	1,000	-5	1,009	5	-4	1,050	17	1,192,000	16	32
17....	Chronic adenitis	13,000	-12	8,381	7	*-43	14,800	124	4,768,000	30	86
18....	Anemic child	10,000	+69	9,390	94	+59	5,900	174	3,080,000	25	65
19....	Cholangitis	18,000	+28	15,653	74	+21	13,000	17	3,640,000	32	80
20....	Pernicious anemia	7,000	+22	10,786	74	+88	5,750	18	832,000	19	58
21....	Pernicious anemia	4,000	-22	3,414	7	-33	5,100	17	1,880,000	29	50
22....	Anemic child	4,000	*-65	3,880	6	*-66	11,300	10	7,440,000	41	75
23....	Pernicious anemia	7,000	-34	6,984	7	-34	10,600	22	3,296,000	25	100
24....	Pernicious anemia	5,000	+5	11,330	7	*+139	4,750	19	2,840,000	21	55
25....	Anemia	12,000	*+97	9,002	84	+48	6,100	16	2,416,000	22	20
26....	Pericarditis	15,000	-4	16,374	8	+5	15,665	15	4,588,000	17	85

TABLE I.—Continued.

Number of Case.	Diagnosis.	Inspection.		Smear-counts.			Thoma-Zeiss.				Hemoglobin.
		Leucocytes per. cu. mm.	Per Cent Error.	Leucocytes per. cu. mm.	Time in Minutes.	Per Cent Error.	Whites.		Reds.		
							Per. cu. mm.	Time in Minutes.	Per. cu. mm.	Time in Minutes.	
27....	Pernicious anemia.....	4,000	-41	3,104	6	*-46	6,733	10	3,120,000	18	85
28....	Pernicious anemia.....	1,500	+5	1,242	6	-13	1,422	16	938,000	14	24
29....	Cancer of stomach.....	12,000	+3	13,192	7½	+13	11,666	11½	1,478,000	17	31
30....	Splenectomy.....	5,000	*-62	10,476	8	-21	13,200	7	4,636,000	14	85
31....	Chlorosis.....	4,000	-26	4,734	6	-13	5,440	10	2,124,000	19	48
32....	Pernicious anemia.....	1,750	-6	1,940	7½	+4	1,866	18	831,000	19	20
33....	Hodgkin's disease.....	6,000	-8	5,432	7½	-17	6,533	8½	5,872,000	16	98
34....	Acetanilide poisoning.....	7,500	-29	8,070	8	-23	10,500	13	3,808,000	22	81
35....	Pernicious anemia.....	1,200	-46	3,182	7	+42	2,240	13	1,110,000	20½	22
36....	Pernicious anemia.....	5,000	-17	8,148	6½	+37	6,000	9	1,486,000	13½	38

37....	Addison's disease	5,500	+22	4,656	6	+3	4,500	10	4,568,000	17	60
38....	Purpura	8,000	-9	7,682	7	-13	8,800	9	5,610,000	17½	100
39....	Pernicious anemia.....	5,000	-14	3,026	5	*-48	5,800	14	2,560,000	11	77
40....	Secondary anemia.....	2,500	-31	1,319	4½	-63	3,600	11	4,272,000	16	37
41....	Pernicious anemia.....	4,500	-17	5,587	5½	+3	5,450	9	2,190,000	17	55
42....	Pernicious anemia.....	7,500	+35	7,217	7½	+30	5,550	10½	966,000	17	34
43....	Pernicious anemia.....	2,000	-40	3,958	5	+19	3,333	12	1,123,000	18	31
44....	Hodgkin's disease.....	4,500	-21	4,423	5	-22	5,700	8	5,712,000	19½	110
45....	No diagnosis.....	5,000	+35	3,259	4½	-12	3,700	11	4,452,000	20½	45
46....	Pernicious anemia.....	7,500	-26	14,589	10	+43	10,200	10	732,600	11	10
47....	Intestinal tuberculosis	15,000	-10	8,303	5½	*-50	16,600	9	5,141,000	20	96
48....	Phlebitis.....	8,000	-25	7,605	5½	-28	10,700	10	5,408,000	14½	110
49....	Pernicious anemia.....	5,000	-2	7,372	6	+45	5,100	8	1,974,000	13½	45
50....	Pernicious anemia.....	3,500	+9	2,949	5½	-8	3,200	11	1,800,000	17	45
51....	Chlorosis	4,000	*-59	9,700	6½	0	9,680	9	2,731,000	17	40
52....	Chlorosis	5,000	-12	4,734	4	-17	5,700	9	3,792,000	13	30
53....	Adenitis	3,500	-43	4,423	5	-28	6,114	12	5,056,000	15	92

TABLE I. — *Continued.*

Number of Case.	Diagnosis.	Inspection.		Smear-counts.			Thoma-Zeiss.			
		Leucocytes per cu. mm.	Per Cent Error.	Leucocytes per cu. mm.	Time in Minutes.	Per Cent Error.	Whites.		Reds.	
							Per cu. mm.	Time in Minutes.	Per cu. mm.	Time in Minutes.
54....	Hemorrhage	3,500	—50	10,243	5½	+48	6,933	8½	2,531,000	17
55....	Anemia	3,500	*—68	8,380	6	—23	10,900	13	1,009,000	21½
56....	Pernicious anemia.....	2,500	*—68	4,423	6½	—43	7,733	10	2,194,000	22
57....	Secondary anemia.....	9,000	—31	9,545	7	—27	13,100	12½	4,902,000	10
58....	Secondary syphilis	5,000	—13	7,915	6 *	+37	5,760	14½	5,043,000	17½
59....	Abscess thigh	18,000	—42	28,402	9½	—9	31,200	6½	4,392,000	18½
60....	Abscess leg	20,000	—43	42,835	12	+22	35,000	8	2,968,000	16
61....	Polycythemia	10,000	*—50	22,426	10½	+12	20,000	9½	8,448,000	17

TABLE II.
Leukemia.

Number of Case.	Kind of Leukemia.	Inspection.		Smear-counts.			Thoma-Zeiss.				Hemoglobin, per cent.
		Leucocytes per cu. mm.	Per Cent Error.	Leucocytes per cu. mm.	Time in Minutes.	Per Cent Error.	Whites.		Reds.		
							Per cu. mm.	Time in Minutes.	Per cu. mm.	Time in Minutes.	
62....	Acute lymphatic	100,000	+6	108,950	19	+15	94,400	15
63....	Chronic myelogenous	1,000,000	+19	Impossible	to estimate thus		840,000	19	2,032,000	29	55
64....	Chronic myelogenous	200,000	-39	324,368	7	-1	328,000	12	3,309,000	19	50
65....	Chronic lymphatic	100,000	-65	220,384	4½	-23	288,000	9	4,616,000	17	85
66....	Acute lymphatic	50,000	-44	81,635	6	-9	89,600	9½	1,146,000	14	20
67....	Lymphatic	30,000	+33	22,116	8	-2	22,600	6	4,476,000	15	92

slide and cover-glass, the shaking of the pipette, and the final cleansing of the apparatus as well as the actual counting and reckoning. It did not include the time used in puncturing the ear and filling the pipette. The average time with the Thoma slide was fifteen minutes; with the Türk slide ten and seven-tenths minutes. The red count acquired an average of twenty-six minutes in the first twenty-five cases, the rule being to count three hundred small squares in two drops. In the remaining cases the time was about seventeen minutes, the rule being to count at least a thousand cells in one drop. Certainly the Thoma-Zeiss method is not particularly difficult or time-consuming, and the Türk slide is a real time-saver.

With smear-counts the results were certainly disappointing. They varied from those obtained by the Thoma-Zeiss apparatus all the way from sixty-six per cent too low to one hundred and thirty-nine per cent too high. The average error was thirty per cent (average plus error 36 per cent, average minus error 26 per cent). The probable error was about twenty-two per cent. In the nine cases marked in the table with the asterisk the error was such as to have, considering the facts of the individual case, considerable importance.

In accounting for these great errors it would seem probable that they depended upon the varying thickness of the films. It also appeared possible that they might depend to a degree on variations in the number of red corpuscles in a unit volume of blood, for in selecting favorable fields one naturally judges by the spreading of these, the most numerous objects in the smear. If this were so one would expect to find the whites overestimated in cases where the reds are low and *vice versa*. Inspection of the tables shows this to be, in a general way, the case, though there are striking exceptions in both directions. This being so, the method, while too inaccurate for anemic cases, might give better results if restricted to those where the reds are normal. Accordingly, Table III. was made up of those cases, twenty-five in number, where the red cells were between four and

TABLE III.
Non-anemic cases.

Number of Case.	Whites by Thoma-Zeiss Method.	Smear-counts.		Number of Case.	Whites by Thoma-Zeiss Method.	Smear-counts.	
		Leucocytes per cu. mm.	Error Per Cent.			Leucocytes per cu. mm.	Error Per Cent.
3.....	10,600	23,019	+117	37....	4,500	4,986	+11
5.....	6,200	8,393	+35	38....	8,800	8,227	-7
6.....	10,800	12,631	+17	40....	3,600	1,413	-61
9.....	8,800	10,138	+15	44....	5,700	4,737	-17
10.....	5,800	6,149	+6	45....	3,700	3,490	-6
11.....	9,133	10,803	+18	47....	16,600	8,892	-46
13.....	15,800	18,365	+16	48....	10,700	8,144	-24
14.....	12,500	13,213	+6	53....	6,114	4,238	-31
15.....	7,600	6,561	-14	57....	13,100	10,221	-22
17.....	14,800	8,975	-39	58....	5,760	8,476	+47
26.....	15,665	17,534	+12	59....	31,200	30,415	-3
30.....	13,200	11,219	-15	67....	22,600	23,684	+5
33.....	6,533	5,817	-11				

six million. A new constant (83.1) was estimated and the leucocyte values figured accordingly. The results are only a little better than before. The average error is twenty-four per cent (average plus error 25 per cent, average minus error 23 per cent) instead of thirty. The individual errors are still large enough to stamp the method as worthless even when thus restricted.

In order to test the accuracy of smear-counts without reference to the Thoma-Zeiss method as a standard, counts by the method in question were repeated in ten cases where the smears had been preserved (see Table IV.). The variations were so great as to further strengthen the case against smear-counting.

TABLE IV.
Duplicate smear-counts on ten cases.

Number of Case.	First Smear-count.	Second Smear-count.	Number of Case.	First Smear-count.	Second Smear-count.
1	6,829	8,381	6	11,795	14,744
2	4,500	5,122	7	11,097	6,130
3	21,495	20,719	8	7,294	9,389
4	17,072	31,583	10	5,742	2,949
5	7,838	3,104	12	28,557	46,870

The average time for making a smear-count was seven and three-tenths minutes, which is to be compared with fifteen minutes, the average for the Thoma-Zeiss method, and ten and seven-tenths minutes for the same with the Türk slide. This time (7.3 minutes) includes that occupied in making the arithmetical calculations, but does not include puncturing, spreading, and staining, since that is generally done anyway for valuable information other than that relating to the number of leucocytes per cubic millimeter. It need only be said that the time needed for making and staining a smear is several times as great as that required for filling a pipette. I certainly fail to see the justification for Einhorn and Laporte's recommendation that this method be used in acute surgical conditions like appendicitis where it is desirable to determine whether the leucocytes are rising or falling from hour to hour, since it is in just such circumstances that the greatest accuracy is needed. Moreover, before one can use this method he must undertake a considerable piece of research to determine the constant for his own microscope. Many a count would have to be made before the brief three or four minutes saved on each one would amount to the time thus expended.

The method of estimating leucocytes by simple inspection of a stained smear without counting at all certainly has the advantage of simplicity and brevity. Only a glance at a few

fields is needed. Allowances may be made for the presence of anemia, for the thickness of the smear, and for other varying factors. In this study the average error in estimates so made was thirty per cent (average plus error 26 per cent, average minus error 31 per cent), which is almost exactly the same as in the smear-counts (see Table I.). The probable error was about twenty-six per cent. The extreme variations from the Thoma-Zeiss count were no greater than they were with the smear-counts, nor was the number of cases in which the error would have had clinical importance.

Simple inspection of the smear, then, is as accurate a way to judge the number of leucocytes as counting fields. It doubtless requires more experience. Certainly it is not for an instant to be thought of as a method by which one can save himself the trouble of learning the technic of accurate counting.

The conclusion seems warranted that while an experienced investigator can, by a study of stained smears, often make a rough approximation as to the presence or absence of leucocytosis, the method is too uncertain for use even in rough-and-ready practical work. Smear-counts give no better results than simple inspection and save but little time over accurate counts with the pipette and counting-slide. There is a real saving of time with the Türk slide as compared with the Thoma.

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REPORT OF A CASE OF OIDIOMYCOSIS.*

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On January twenty-first, 1907, I saw, in consultation with Dr. Geo. E. Reed of Brooklyn, a patient whose history is as follows: F.N., aet. twenty-seven years, policeman by occupation, of unusual physical development (six feet three inches in height; weight, two hundred and seventy pounds). The man's health had always been good; the only thing in the previous history that might possibly have some bearing upon the etiology was the fact that, while bathing in the early part of August, he cut his left foot with a clam shell. The foot swelled and was quite painful for the next few days, without, however, detaining him from his regular work. On about the first of December he was taken with a severe attack of pain in the lumbar region, which he regarded as lumbago and which kept him confined to the house. Two to three days later he began to have severe pain in the dorsal region of the left foot, which became markedly swollen, the swelling apparently starting over the second or third meta-tarsal bone and quickly involving the entire dorsum of the foot; after a few days it showed signs of fluctuation, and at the end of ten days a small sinus appeared between the great and second toes, from which there exuded dirty pus-like material; the skin, while tense and distended, was not reddened. About a week later a similar lesion developed on the dorsum of the other foot and, on the outer aspect of the right thigh, just above the knee, two small flat papillary tumors appeared in the skin itself. These were irregularly circular in shape, depressed in the center, with indurated edges, slightly elevated above the normal skin. They became quickly covered with crusts which, on removal, showed a small collection of pus-like material in the center. The patient states that he often

* Received for publication April 15, 1907.

scratched and picked around these ulcers and later scratched his face. Whether as a result of auto-inoculation or not, about a week later similar small papillary tumors developed in the face, the number and character of which are well shown by the accompanying cut. The lesions upon the face were larger and more protuberant than those just described upon the thigh, projecting about one-quarter to one-third of an inch above the surrounding surface; they were reddened and indurated at the base, soft in the center and quickly covered by thick scabs or crusts. These could be easily picked off and always showed underneath a few drops of yellowish pus. Almost simultaneously with the skin lesions described, there developed numerous subcutaneous tumors widely distributed over various parts of the body, but chiefly in the thighs and arms. These tumors varied from the size of a hazelnut to that of a small egg; they always began subcutaneously or in the deep layer of the skin, appearing first as smooth, spherical tumors of moderately firm consistence, scarcely raised above the level of the skin. As after a few days they increased rapidly in size, they became much more protuberant, the skin showed attachment in the center; they soon showed signs of fluctuation and, after a few days, the entire tumor seemed little more than an abscess cavity. If left to themselves they usually broke after a week or ten days, discharging a dirty, cream-colored material. Coincidentally with the appearance of the first tumors, the patient began to have a dry hacking cough which is almost constant and has persisted steadily up to the present time, refusing to yield to any form of treatment. The patient's general health deteriorated very rapidly; within the first three weeks he lost sixty pounds in weight. Two weeks after the beginning of the symptoms, one of the small subcutaneous tumors was removed before it ruptured and microscopic examination was made by Dr. J. M. Van Cott, of Brooklyn, who pronounced it giant-celled sarcoma.

The patient was admitted to my service at the General Memorial Hospital on January twenty-third, three weeks after the beginning of the disease and two days after I first

saw him, at which time the condition was as I have just described. In addition to the two superficial papillary skin lesions on the right thigh as mentioned, there were five or six similar lesions in the face and about twenty of the subcutaneous tumors in different parts of the body. The course of the disease since his admission to the hospital has been briefly as follows:

Photographs and drawings were made three days after his admission, or at the end of the third week of the disease. On January twenty-fifth, after carefully sterilizing the skin and under all possible aseptic precautions, I opened several of the subcutaneous tumors, and Dr. S. P. Beebe, of the Huntington Cancer Research Fund, made cultures upon a large variety of media, and several dogs and guinea-pigs were inoculated with the material. As none of the culture tubes showed any growth at the end of four days they were thrown away. A section of the wall of one of the tumors was examined by Dr. James Ewing, and although the general appearance was strikingly like that of a giant-celled sarcoma, one or two round bodies characteristic of the blastomycosis organism were found, and the diagnosis of blastomycosis was made. In the opinion of Dr. Ewing, the giant-cells were more like foreign body giant-cells than those seen in sarcoma. This organism is of much slower growth than pyogenic bacteria, seldom showing growth before the fourth day, which accounts for the fact that our previous cultures were considered sterile. I thereupon opened several other tumors, also the large swelling upon the right foot from which about four ounces of material of the consistence of cream, but much darker in color than the fluid removed from the subcutaneous tumors, was evacuated. New cultures were made by Drs. Beebe and Tracy and on about the fourth day they began to show pure cultures of the organism. The accompanying cuts show its appearance at different stages before and after budding. Animal inoculations were made with the pure cultures with the result as shown by Dr. Tracy's report:

PATHOLOGICAL REPORT BY DR. MARTHA TRACY OF THE
HUNTINGTON CANCER RESEARCH LABORATORY, GEN-
ERAL MEMORIAL HOSPITAL.

The tissue sent for examination was cut from the wall of a subcutaneous abscess. It was of a rather pale pink color and medium firmness. Fixation was in Müller-formol and staining with hematoxylin and eosin.

Microscopic examination shows tissue composed of rather loose stroma and abundant round cells. Very many of the cells are polynuclear leucocytes. Others, less numerous, are larger, more or less epithelioid in type with large vesicular nuclei. Large giant cells are also present. These are particularly abundant in a section from the original abscess in the foot. The giant cells are of a characteristic type, containing six or more large nuclei, centrally placed. Many new formed blood vessels are present, and here and there collections of free red blood cells. The whole appearance is typical of an active inflammatory reaction.

Throughout the section, and often within giant cells, are seen the spherical forms of the oidiomyces. The bodies are granular and their nuclei take the hematoxylin stain. They contain refractile points, and the double refractile contour is conspicuous. Some of the giant cells contain four or more of the organisms of various sizes.

No endogenous cell division is observed, but occasional budding forms are seen.

Sections from lesions involving the skin show considerable dipping down into the corium of cords of epithelial cells. In sections of tissue from an experimental subcutaneous lesion in a dog the microscopic appearance is similar, save that the giant cells are less numerous. Rather sharply limited aggregations of leucocytes are noted, about the periphery of which the spherical organisms are numerous. Here several budding forms are found.

Pus from the abscesses in moist smears show very many spherical and budding forms of the organism. A hanging drop of the pus in physiological salt solution showed

mycelial growth in forty-eight hours. Smears from the pus of the original abscess in the foot and from the secondary lesions were made on glycerine agar, glucose agar, and potato; growth was evident in the potato tubes and in the agar tubes of alkalinity + 8 (phenolphthalein) after four to five days. In tubes slightly more alkaline three weeks elapsed before the colonies appeared. After the first appearance the growth was quite rapid at room temperature. The cultures on glucose agar were somewhat more luxuriant than those on glycerine agar.

The appearance of the growth is typical. White, pin-point raised colonies appear which increase on the surface of the agar and extend down into it to a slight extent. The colonies are very brittle, and mycelial threads are thrown out on the free surface.

Subcultures grow more rapidly than did the original, and seemed but slightly retarded by low temperatures, even that of the ice-chest.

In broth the growth formed a semi-opaque mobile sphere of the size of a walnut in a month.

Cultures from the patient's blood were negative.

Rabbits, guinea-pigs, white mice, and dogs were inoculated from the pus. Only two of the animals gave positive results. One mouse inoculated intraperitoneally died of generalized infection in a week. Lesions showing the organism in the sections were found on the peritoneum, in the liver, and kidney. Cultures from these organs failed to grow.

One dog developed subcutaneous abscesses resembling closely the secondary lesions in the patient. From the pus the organisms were successfully grown in the hanging drop and on glucose agar.

The mycelial formation, which took place in all the cultures, determines the position of the organism in the class of oidiomyces, as distinguished from the blastomyces or true yeasts which multiply only by budding.

A more elaborate culture study of the organism has not yet been completed.

Dr. Beebe also made a number of blood cultures, but these were found uniformly sterile.

During the first two weeks of the patient's stay at the hospital, while the possibility of the condition being sarcoma was entertained, he was given ten injections with the mixed toxins of erysipelas and *Bacillus prodigiosus*; the highest dose was six millimeters. Only moderate reactions followed, his temperature never going higher than 103° F. He had been running an irregular temperature ever since his trouble started, same ranging from 97° to 102° F. After the diagnosis of blastomycosis was made I put him upon large doses of iodide of potassium, the value of which in this disease has apparently been demonstrated by Bevan and Hyde in the group of cases observed in or about Chicago. The dose was increased steadily, until he was receiving two hundred and fifty grains a day, but the treatment apparently had not the slightest effect in retarding the progress of the disease. The patient continued to lose weight steadily, new lesions continually appeared, the cough persisted and hemoglobin rapidly fell. Examination of the urine at the time of entrance showed a specific gravity of 1,030; acid reaction; clear amber color; a trace of albumin. There have been no albumin nor casts since. The specific gravity has steadily fallen; the last examination on April seventh showed 1,008, straw color, no albumin, no casts. Frequent examinations of the blood have been made.

The first, January 24, showed:	
White blood cells	18,000
Red blood cells	4,000,900
Hemoglobin	85-90 per cent
January 27:	
White blood cells	18,200
Polynuclear	75 per cent
Small lymphocytes	20 per cent
Large lymphocytes	3 per cent
Eosinophiles	1 per cent
Giant cells	½ per cent

Two other counts made the same day showed practically the same result.

February 4:	
White blood cells	10,000
Red blood cells	3,000,840
Hemoglobin	75 per cent

February 16:	White blood cells	31,000
	Red blood cells	3,480,000.
	Hemoglobin	50-55 per cent
	Polymorphous	76 per cent
	Small lymphocytes	21 per cent
	Large lymphocytes	3 per cent
February 26:	Red blood cells	3,000,160
	Hemoglobin	55 per cent
	White blood cells	21,800
	Lymphocytes	9 per cent
	Mast cells	1 per cent

March 23, 1907: There is a marked decrease in the number of red blood cells and hemoglobin and a great increase in the polynuclear cells:

Red blood cells	2,000,250
Hemoglobin	30-35 per cent
White blood cells	30,000
Polynuclear	92 per cent
Small lymphocytes	5 per cent
Large	2 per cent
Mast cell	$\frac{1}{4}$ per cent

The temperature at this time ranged between 97.8° F. and 102.8° F. Some days, however, it would not rise above 99.5° F.

As the patient's condition had been steadily getting worse under the iodides, they were discontinued on April first and, purely as a matter of experiment, the toxins resumed, one minim being given on April first and the dose has been increased by one minim a day since.

On April 3 the blood count shows:	Red blood cells	3,240,000
	Hemoglobin	40
	White blood cells	24,400
	Polymorphous	88 per cent
	Small lymphocytes	7 per cent
	Large lymphocytes	2 per cent
	Eosinophiles	3 per cent

being a considerable improvement over the previous examination.

At present, April eighth, he has sixty-five tumors or lesions distributed throughout the body as follows: Face and

neck, fifteen, thirteen of which have been opened; abdomen, seven, four opened and curetted; legs and thighs, twenty-four, of which twenty-two have been opened and curetted; arms, eleven, eight opened; chest, two; back, six, two of which have been opened. The largest one of all of these, excepting the lesion in the dorsal region of the foot, is one that has recently formed over the sternum, being about the size of a goose egg. It has already softened and will be opened shortly. The lesions upon the face have nearly disappeared under the treatment with carbolic acid, three to four applications having been made in the following manner: a saturated solution of Schering's carbolic was applied by means of cotton to the face lesions, the acid being allowed to remain for one minute, then the effect was counteracted by alcohol. The skin lesions have almost entirely disappeared, leaving, however, well-marked reddened scars.

The site of the initial lesion in this case was, to my mind, very probably the lungs, although there is a possibility of the infection's having entered the blood current through the wound in the foot in August, starting a primary lesion in the lung, from which the general infection spread.

A careful study of the cases of so-called blastomycosis thus far reported, as well as those of coccidioidal granuloma, leads me to believe that clinically my own case belongs to the latter rather than to the blastomycosis group. The latest paper on this subject is that of Philip King Brown of San Francisco (in the March second, 1907, issue of the Jour. of the Am. Med. Ass'n) containing a very complete report of one case of his own, two additional, one very complete, the other somewhat doubtful, together with a summary of sixteen previously published cases. The clinical history and course of the disease in my own is strikingly identical with that of the case so fully reported by Brown. In his, as in my own, the subcutaneous swellings were primary and the cutaneous lesions secondary and unimportant. In his there was a large swelling over the dorsum of the right hand, while in mine the dorsum of the foot was affected. In both cases

there was absence of glandular swelling. There was no redness, or tenderness or edema of the overlying skin. The cutaneous lesions were likewise identical, beginning with papillæ, rapidly developing into papillary tumors, circular in shape and covered with crusts. In Brown's case the range of temperature is practically the same as in my own, 99° F. in the morning, often running to 102-3° F. in the evening. The blood examinations in his case, likewise, were similar to mine, being:

Reds	2,500,000
Whites	13,000
Polymorphonuclears	79 per cent
Small	17 per cent
Large	2 per cent
Eosinophiles	2 per cent
Hemoglobin	50 per cent

In addition, Dr. Mary Halton, of San Francisco, who made the autopsy in Dr. Brown's case and had an opportunity of studying the cultures, has just made a careful examination of our own slides and cultures and pronounced them identical with those of Brown's case.

Our cultures and slides were submitted to Dr. Welch, of Johns Hopkins, who is unwilling to state definitely whether the organism is of the coccidioidal or blastomycotic type. He says that the two modes of multiplication of the tissues is the chief distinguishing mark between the San Joaquin Valley parasite and that observed in other parts of this country (except Wolbach's case in Boston, which is coccidioidal). He adds that it would be interesting (and may be true) if both forms of the parasite produce identical lesions and symptoms, in certain cases, although he was under the impression that the coccidioidal disease was more like tuberculosis. In the specimens which he had seen of the coccidioidal disease the internal lesions were astonishingly like tuberculosis.

Specimens of our case have been recently sent to Wm. Ophüls, of San Francisco, as the highest authority on the subject. Up to the present time I believe that no cases of

coccidioidal granuloma have been described in people who had not been in California, except the very first case observed by Wernicke (*Centralbl. f. Bact.*, 1892) and the case of Wolbach of Boston. The first case in California was described by Rixford (*Occidental Med. Times*, 1894, viii, 326). Another similar one under the care of Drs. Thorne and Robinson (Thorne—*Occidental Med. Times*, viii, 703) was seen by him the same year.

Thus far there have been reported forty-two cases of blastomycosis or blastomycotic dermatis, of which thirty-two were local and ten disseminated. Of coccidioidal granuloma or oidiomycosis there have been eighteen cases reported.

As to the supposed distinguishing features between the two groups of cases, it may be said that coccidioidal granuloma is almost invariably progressive, with marked tendency to generalization by blood and lymph, the secondary skin lesions being due to emboli. Generalized infection is the rule. Only one patient is known positively to be alive. In this case the primary lesion was in the foot, which was promptly amputated. In these cases, iodide of potassium is practically of no benefit.

In cases of blastomycosis, the disease is usually local and progress is slow, and the tendency to generalization slight. Thus far there has been observed only one case with generalization, which died, although iodide of potassium had been followed by improvement.

In the cases of coccidioidal granuloma, the site of the initial lesion was in the skin in five; internal (chiefly lungs) in twelve; unknown, one.

As regards the residence of the eighteen cases so far reported, fourteen are positively known to have resided in the San Joaquin Valley; one, negative; three, the place of residence is not known.

Dr. James Ewing, professor of pathology at Cornell University, who has made a careful study of the organism as well as the sections from the tumors, from our case expresses his opinion as to its nature, as follows:

"It is impossible in our present state of knowledge to give the final classification of the organism in this case; it can only be done in a general way. The three groups among which it lies are the blastomycetes, the oidia, and the hyphomycetes. The blastomycetes possess large cells with definite membranes. They multiply by budding, and never produce mycelia. Your organism has a definite membrane, but produces abundant mycelium and hence is not a blastomycetes. The hyphomycetes produce mycelia, and hyphæ and sporangia. Your organism has no sporangia and hence is not in this class. The oidia lie between these classes, as they multiply by budding and produce mycelia. Your organism multiplies by budding in the tissues and produces abundant mycelium in cultures. Sporangia have not yet been observed in it. Some organisms of this group produce very little mycelium in cultures and that very slowly. There seem to be a great many species in the group, some in which budding is more prominent, others in which mycelium formation is more pronounced. Unfortunately, these characters are not stable, hence the difficulty of classification. Therefore Ricketts makes three classes, (1) Blastomycetoid, (2) Oidium-like, (3) Hyphomycetoid.

"Wolbach's organism is peculiar in that it does not bud in tissues, but multiplies by endogenous sporulation producing large cysts with many spores. Nevertheless, it produces mycelia and sporangia in cultures. It is, therefore, hyphomycetoid. Your organism is quite different from Wolbach's, producing no endogenous spores, budding freely, but not, so far, producing sporangia. It may, therefore, be placed for the present in the intermediate group of true oidia. Much further study will be required before it can be finally classified.

"APRIL 25, '07: The patient has grown worse very rapidly since the last note, and will probably not live longer than one or two weeks. A large number of new tumors have developed, and there has been a recrudescence of those upon his face. A purulent discharge from the rectum shows the organism, also the sputum."

Copy of Dr. W. Ophüls' report:

SAN FRANCISCO, CAL., April 11, '07.

Blastomycosis case.—“In the sections the organism is considerably smaller than the one which we find in California. As you say they show budding and no endosporulation. The macroscopic appearance of the culture is exactly like that of the organism of the coccidioidal granuloma, but microscopically this also shows some differences. The tryphæ show lateral budding in places which I have never observed in my cultures, there are many spherical and pear-shaped bodies which also do not occur in them, and there is complete absence of the peculiar angular spores. These latter, however, do not form in all cultures.

“On the whole, the organism is more like some of those described from Chicago. I believe, however, with Ricketts, that all these organisms belong to the same group. It may be preferable to classify them with the blastomycetes rather than with the oidia as Ricketts does.”

[Thanks are due to Dr. William J. Elser and Dr. Frank M. Huntoon for the successful cultivation of the organism and for permission to report their results, and to Dr. D. H. M. Gillespie for careful search of the literature of the subject.]

APRIL 6, 1907.

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EXPLANATION OF PLATES.

PLATE XVIII.

FIG. 1. — Low power appearance of section of granulation tissue from abscess wall. Many giant cells containing blastomyces. Camera lucida, x 500.

FIGS. 2, 3, 4, 5. — Giant cells from blastomycetic granulation tissue — abscess wall. One to very many spherical organisms within the cells. Camera lucida, x 1,000.

FIG. 6. — Showing development from spherical forms to long mycelial threads. Hanging drop preparation. Camera lucida, x 1,400.

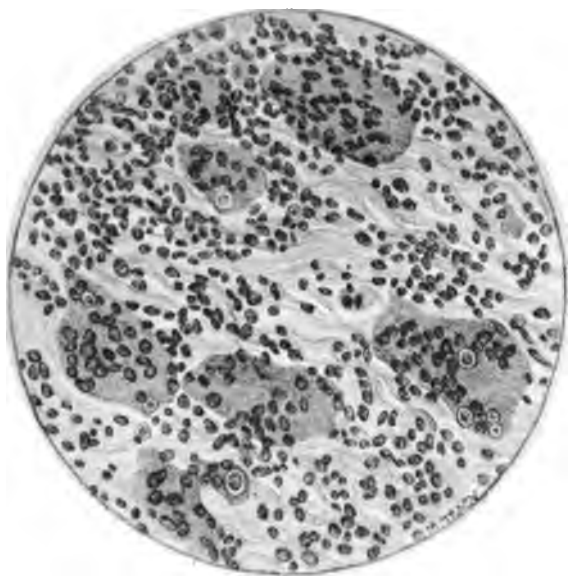
PLATE XIX.

Views of some of the skin lesions.

PLATE XX.

FIG. 1. — Blastomyces in giant cell. x 1,000.

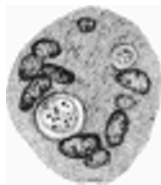
FIG. 2. — Blastomycetic granulation tissue. x 180.



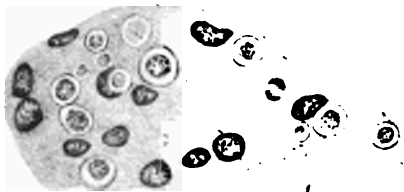
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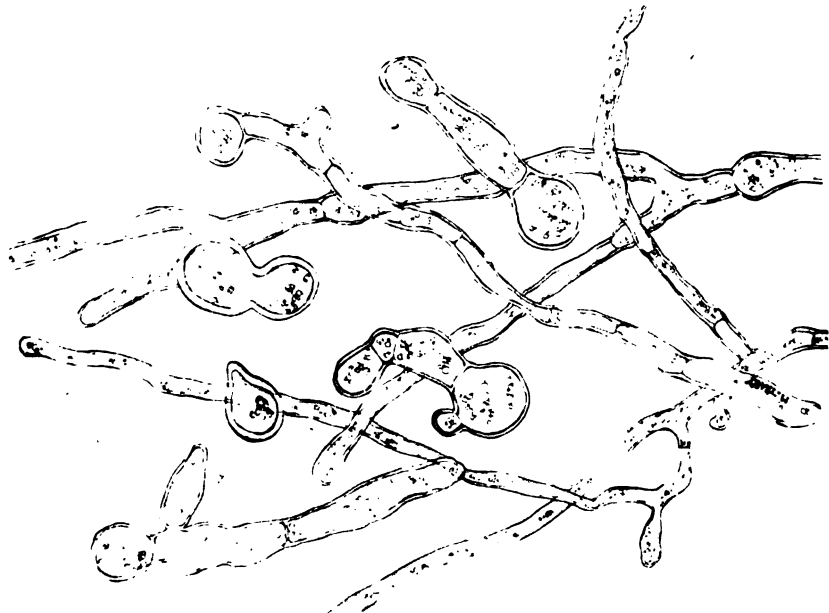
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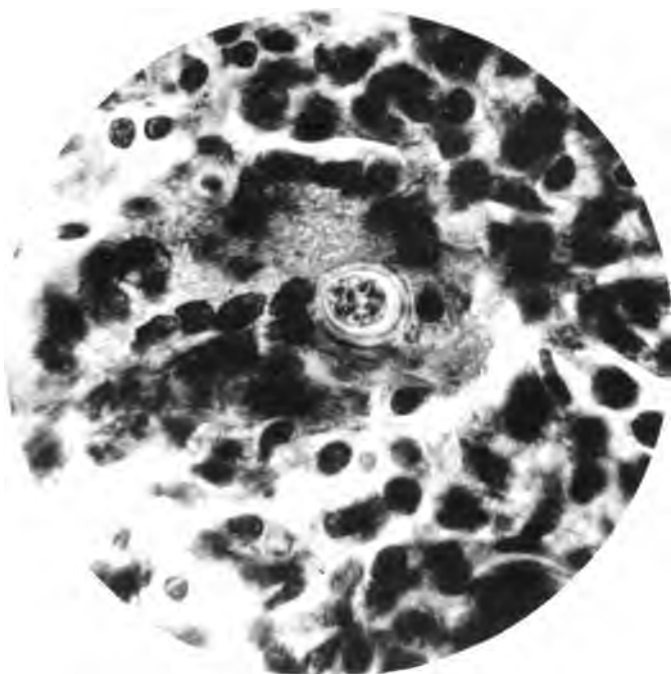
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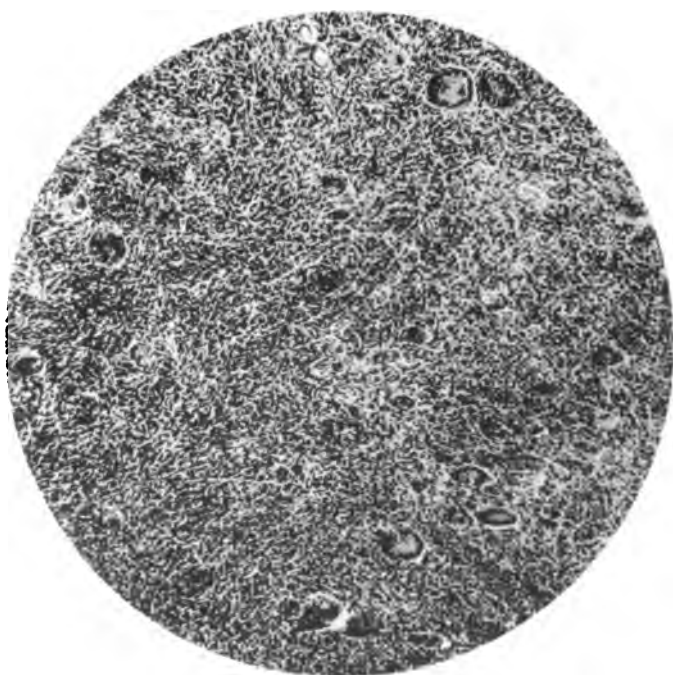
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2



1



2

ABSORPTION FROM THE PERITONEAL CAVITY.*

PART VIII.—ABSORPTION OF TYPHOID BACILLI IN THE
IMMUNE ANIMAL.

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For the purposes of this investigation rabbits were immunized with cultures of typhoid bacilli previously killed by heating to 60° C. for one hour. The immunizing injections were at first made intraperitoneally, but it was soon found that by this method a subacute to chronic inflammatory process was set up in the peritoneal cavity which afforded a non-specific protection. The immunizing inoculations were then made subcutaneously or intravenously, and after five cultures subcutaneously, or four or five inoculations of small doses intravenously, the rabbits were inoculated intraperitoneally with living typhoid bacilli, and treated according to the methods described in previous sections of this work. Rabbits thus immunized, however, showed such slight differences from the normal animals that it was supposed they had not been sufficiently treated, and the immunization for the next series of rabbits was extended over a longer period. The animals dealt with in the following tables, and marked S.C. in the first column, received altogether ten killed cultures of typhoid bacilli subcutaneously, fifteen inoculations being given, extended over a period of fifteen weeks. For the first ten weeks one-half of a culture was inoculated each week, and during the last five weeks one culture each week. Nine or ten days after the last inoculation the rabbits received intraperitoneally one-half of a culture of living typhoid bacilli and were then sacrificed at varying intervals.

The rabbits marked S.C. and I.V. in the first columns of the Tables I. to VIII. were treated in precisely the same way, except that instead of receiving five subcutaneous inoculations of a whole culture each time, they received, for the last

* Received for publication April 8, 1907.

five doses, intravenous inoculations of about one-eighth to one-tenth of a culture each week. It was found that this dose was about as much as the animals could stand intravenously.

METHODS. — The methods followed in carrying out these experiments have been described in detail in previous communications, but may be briefly recalled at this point.

DOSE. — Intraperitoneally. One-half of a culture of living typhoid bacilli, estimated at about four to five thousand millions of bacilli.

BLEEDING. — At varying intervals after the intraperitoneal inoculation the rabbit is bled to death from the carotid, one cubic centimeter of blood being run into one cubic centimeter of bile, the rest of the blood allowed to run into tubes, and the serum collected next morning. The bile-blood mixture is plated out in fractions and the number of bacilli per cubic centimeter estimated from the colonies.

WASHING OUT. — The peritoneal cavity is washed out with four hundred cubic centimeters of salt solution and, from dilutions of the wash water, plates are made from which the number of bacilli remaining alive and free in the cavity is estimated.

ORGANS. — The spleen is taken as a unit and rubbed down in fifteen cubic centimeters of salt solution. Portions of the same size as the spleen are taken of the liver, lung, and kidney, and each rubbed down in the same way. The marrow of one thigh bone is also taken out and rubbed down. One cubic centimeter of each of these pulps is plated out, and the number of colonies on the plates is given in the tables. In the tables the results with lung and kidney have been omitted since they do not present any points of special interest. The liver and spleen are the best indicators of the extent to which the organism has been invaded by the

bacilli. The two upper anterior mediastinal lymph nodes are picked out and rubbed down in fifteen cubic centimeters of salt solution. Fractions of the suspension are plated out and from the colonies an estimate is made of the number of bacilli in the nodes, the estimated totals being given in the tables.

Six immune rabbits were sacrificed at each interval of time chosen, and the results are recorded in Tables I. to VIII., the figures for the spleen being taken to determine the position of each rabbit in the table. It will be observed that when the figures are high for the spleen they are also high for the other organs. This general rule obtained also for the normal animals discussed in Part III. An apology is perhaps due for the large number of tables appended, but it seemed desirable to present as clearly as possible the considerable amount of material gathered, before attempting to discuss the significance of the results obtained.

EXPLANATION OF HEADINGS IN TABLES I. TO VIII. —

Column 1. The inoculations. Subcutaneous throughout, or subcutaneous followed by intravenous, as already explained.

Column 2. The weight of the rabbit.

Column 3. Peritoneal wash. The estimated total number of bacilli alive and free in the peritoneal cavity.

Columns 4, 5, 6. The number of colonies on the spleen, liver, and marrow plates, respectively.

Column 8. The estimated total number of bacilli per cubic centimeter in the blood.

Column 9. The bactericidal power of the fresh serum. Fresh normal rabbit serum will kill about one million typhoid bacilli per cubic centimeter. As a rabbit becomes highly immunized its serum loses all bactericidal power. Column 9 shows that in almost every instance the fresh serum of the immunized rabbits has no bacteriolytic properties, thus indicating a high degree of immunity. The exceptions lie altogether among those rabbits which received subcutaneous inoculations throughout.

Column 10. The agglutinating value of the serum varies

within wide limits, ranging from 1 to 2,500 up to 1 to 50,000, but for the most part is about 1 to 10,000.

COMMENTS ON TABLES I. TO VIII. — Although, as with the normal animals previously tested, there is great irregularity from one rabbit to another, yet it is obvious that in generality there is an immediate and overwhelming rush of bacilli throughout the organs, to which they are carried by the circulation. In one hour there is a very marked decline in the numbers in the organs followed thereafter by a more gradual decrease, until in forty-eight hours there are very few bacilli left alive.

Tables IX. to XIII. show the numbers of bacilli in the individual organs at different intervals. There is at first a tendency towards a greater deposition in the liver than in the spleen. After four and six hours there is very little difference and by sixteen hours the positions of the two organs are reversed, the spleen now containing relatively far more bacilli than the liver. The decrease in the spleen is comparatively slow, and even in forty-eight hours the organ contains a considerable number of bacilli.

In the case of the blood (Table XI.) the decline after the initial rush is very rapid, the bacilli having practically disappeared in twenty-four hours.

The mediastinal lymph nodes (Table XII.), through which the bacilli pass on their way to the circulation, show some analogy to the figures for the spleen, the decrease being gradual and the nodes still containing large numbers of bacilli in forty-eight hours.

The bacilli left alive in the peritoneal cavity, Table XIII., show a decrease somewhat analogous to that which takes place in the liver. There appears to be no tendency towards a progressive peritonitis, and the difficulty of setting up a localized peritonitis in the experimental animal has often been remarked upon.

TABLE XIV. AVERAGES. — Since there is so much irregularity in the numbers of bacilli from one animal to another,

a table of averages is not of much value, but by leaving out the first rabbit in each table, as representing an exceptionally large number, and the last as representing an exceptionally small number of bacilli, an average can be struck from the middle four rabbits which affords approximately an idea of the results one may ordinarily expect. Such averages worked out to round numbers are given in Table XIV., and from this table it can be seen at a glance how rapidly and progressively the bacilli disappear from the blood, liver, and peritoneal cavity, whereas in the spleen and lymph nodes the decline is much more gradual. It appears that there is a tendency towards accumulation of the bacilli in the lymphoid organs. So far as the lymph nodes are concerned, there seems to be a tendency towards increase of the bacilli in four and six hours over the figures for one and two hours. This increase can probably be accounted for by supposing that the bacilli at first are able to pass through the lymph nodes without much opposition, but after a few hours the nodes have become awakened to activity, the sinuses are filled with phagocytes and thereafter they are better able to intercept further supplies of bacilli from the peritoneal cavity.

COMPARISON WITH THE NORMAL ANIMAL. — It is a remarkable fact that all the above remarks on the immunized apply equally well to the normal rabbit, with the exception of the fate of the bacilli in the peritoneal cavity itself. This exception will be discussed later, but an examination of Tables XV. to XVIII., giving comparisons between the normal and immune rabbits, so far as regards the spleen and liver, show that there is no difference whatever in kind, and practically no difference in degree of bacteriolysis. The numbers of bacilli found in the spleen and liver of the immunized animal appear to be on the whole somewhat greater than in the normal one, but it must be noted that the spleen of the immunized animal is almost always larger than normal, and since the spleen is taken as the unit of size, the portions of liver taken from the immunized rabbit would also be somewhat larger than those from the normal one.

Allowing for this difference in the size of the pieces taken, there would probably be no appreciable difference in the figures whatever. There appears, however, in the immunized animal to be a slightly greater tendency towards an accumulation of the bacilli in the spleen, and a correspondingly more rapid depletion of the liver than is the case with normal rabbits.

GENERAL DISCUSSION.— It has become obvious in the course of these experiments that the bacteriolysis is no greater in the immune than in the normal animal, yet the immunized rabbit is certainly on the whole better protected against infection by living typhoid bacilli than the normal rabbit.

RELATIVE SUSCEPTIBILITY OF THE NORMAL AND IMMUNIZED RABBITS.— Among the normal rabbits the mortality has been about fifteen per cent, but of seventy-five immunized rabbits only two succumbed to the final intraperitoneal injection. One, an Angora rabbit, died suddenly in convulsions about one hour and a half after the inoculation. Angora rabbits are delicate animals and with this exception have not been used. The second was a small rabbit which did not thrive and was sacrificed six hours after the inoculation when in a moribund condition. It was found to be swarming with bacilli and the results are not included in the tables. A few of the immunized rabbits showed an initial drop in temperature, followed by a rise, but the great majority were not affected in the least. On the other hand, with the normal animals the initial drop accompanied by obvious ill effects was the rule.

THE ENDOTOXINS.— Since the immunized animal is not specially or specifically bacteriolytic, it might be supposed that the immunity resides in increased tolerance to the toxic products of the bacilli, but this explanation is negatived by the fact that rabbits do not become more tolerant to killed bacilli inoculated intravenously, although occasionally there may appear to be a slightly increased resistance. It is,

however, difficult to judge of this point, since both normal and immunized rabbits vary greatly in their susceptibility.

After the usual intravenous inoculation of one-eighth to one-tenth of a killed culture the rabbit becomes very sick in about an hour and a half. It may then die suddenly in convulsions, or it may become semicomatose and linger for a few hours until death ensues. Death, however, is exceptional, and in the great majority of cases there is rapid recovery, the animal appearing to be quite well again in a few hours. Now these symptoms occur in the rabbit which has already received several similar injections, just as with one which has not been injected previously. The indications are that the dead bacilli are rapidly broken up with liberation of endotoxins, which then act upon certain susceptible nerve centers, and the nerve centers of the immunized rabbit appear to be nearly if not quite as susceptible as those of the normal one. The fact that in the immunized animal there is little or no increased tolerance to endotoxins seems to be very generally recognized by those who have studied the subject in detail. Pfeiffer and Wolff, among numbers of other authors, hold to this view very strongly.

Vaughan, indeed, has succeeded in separating a toxic principle of the colon bacillus from the bacterial substance, and finds that guinea-pigs, carefully immunized with the extract, acquire some tolerance to the toxin. But the tolerance is not marked, and, moreover, it is doubtful how far Vaughan's toxins, extracted by rather drastic methods, are comparable with the endotoxins as they exist in the bacilli themselves.

It seems probable that the increased resistance to living cultures of the immunized rabbit is mainly, if not entirely, due to the increased phagocytic power of its wandering cells, both macrophages and microphages, but principally the former.

PHAGOCYTOSIS BY MACROPHAGES. — The normal peritoneal cavity contains few or no polynuclear cells, except of the feebly phagocytic eosinophile type. But there are great

numbers of large mononuclear cells present in the fluid, and it is these cells which are primarily active in disposing of foreign matter, such as bacilli, introduced into the peritoneal cavity. The macrophages immediately take up large numbers of bacilli, and are carried with their burden to the omentum, upon which they are caught up and into the tissues of which they gradually penetrate. But apart from the macrophages floating free in the cavity, there are a vast number of them in the milky spots of the omentum itself (Parts IV. and V.). These macrophages of the milky spots are found almost immediately after injection to contain large quantities of bacilli, and the only reasonable explanation of this phenomenon seems to be that the cells send out into the cavity long protoplasmic processes (pseudopods), which waving free in the fluid pick up the bacilli and withdraw them into the tissues of the milky spots (see Part V.).

The best time for examination of the omentum is within a few minutes after the intraperitoneal injection, when both the macrophages on the surface and those in the milky spots are found to be filled with still intact bacilli. Later on the bacilli usually become pale staining and granular, more particularly in the cells of the milky spots, and the picture presented is not so clear. When examined at this early stage after inoculation there can be no doubt that the macrophages of the immunized contain far more bacilli than those of the normal rabbit. The actual number of bacilli in the cells is so great that it is impossible to count them and thus obtain a reliable opsonic index, but the cells of the normal animal may contain twenty to fifty, and those of the immunized one may be so packed with bacilli that their number must be reckoned in hundreds. Now it is evident both from their histological structure and physiological properties that the milky spots of the omentum are lymphoid structures, and we may reason from this that the processes as regards the disposition of bacteria are very similar in other lymphoid organs, *i.e.*, the lymph nodes and spleen. After intraperitoneal injection of inert particles or bacteria there is an immediate rush of the particles to the blood, principally, if

not entirely, by way of the anterior mediastinal lymph trunks and nodes. It has been shown by Torrey and myself (Part III.) that immediately after injection of lamp black, the particles are found lying free in the lymph sinuses and efferent ducts of the anterior, mediastinal lymph nodes. The nodes are overwhelmed, so to speak, by the sudden inrush of multitudes of particles, and are unable to resist their passage. After a few hours the macrophages of the lymph nodes actively multiply, and the lymph sinuses become blocked with these cells, each one of which has gorged itself with particles, and the great majority of particles which thereafter reach the blood current or organs must arrive enclosed in phagocytes and not in a free condition. It is probable that after injection of typhoid bacilli into the immune animal the phagocytes of the lymph nodes are able to englobe more bacilli than is the case with the normal animal, so that proportionately more of the bacilli are carried about in the circulation and organs enclosed in macrophages than is the case in the normal rabbit.

With regard to the spleen, Dominici has shown that after experimental inoculation of typhoid bacilli, certain plasmodial masses, which occur normally in that organ, are roused to activity. There is rapid proliferation, and a splitting up of the plasmodial masses into cells, which take on the characters of macrophages and are discharged in immense numbers into the lymph sinuses and channels of the spleen. In immunized rabbits the spleen is almost invariably larger and darker than normal and appears to be in a condition of hyperactivity, no doubt indicating a superabundance of macrophages.

We are led, therefore, to the general conclusion that shortly after injection of bacilli into the peritoneal cavity of the immunized animal a much larger proportion of the bacilli have become enclosed in macrophages than is the case with the normal rabbit, and that as the bacilli contained in the macrophages are broken up, the endotoxins liberated from the bacilli are neutralized by the cells, or at any rate prevented from becoming absorbed at various susceptible foci.

Observations on the omentum seem to indicate that the bacilli in the macrophages may be very slowly digested. The macrophages connected with the milky spots often appear to possess much greater digestive power than those macrophages which are merely caught up and lie on the surface of the omentum. The bacilli in the cells of the milky spots may rapidly swell up and become granular, whereas those in the surface macrophages often remain intact for some hours and may even show signs of growth in the interior of the cells at four and six hours after inoculation, if there has been up to that time little or no polynuclear leucocytic reaction in the peritoneal cavity. This persistence of intact bacilli in the macrophages may account for the persistence of living bacilli in the organs of the immunized rabbit. Although there is a larger proportion of bacilli enclosed within macrophages in the immunized than in the normal animal, yet on plating out the organs of each there is little or no apparent difference, because the bacilli are not killed more rapidly in the one case than in the other. But the difference becomes apparent in the effects of the toxic products of the bacilli. In the normal animal the endotoxins are largely liberated directly into the body fluids, whereas in the immune animal they are chiefly liberated into the cell plasma of a macrophage and have consequently less power for harm. It must be admitted that there is not experimental proof of every suggestion offered, but the experiments so far as they carry us at present point to this hypothesis as being the most probable explanation of the phenomena observed.

It may be remarked here that in the occasional instances where rabbits appear to become somewhat more tolerant to repeated doses of killed bacilli, the increased resistance may be due to the fact that the macrophages of the animal in the course of immunization rapidly take up a larger proportion of the dead bacilli than those of the normal animal. The mechanism of such protection would be quite similar to that suggested for immunity against the ill effects of living bacilli introduced into the peritoneal cavity, and no increased

tolerance to the endotoxins on the part of the susceptible cells of nerve centers need be implied.

PHAGOCYTOSIS BY THE POLYNUCLEAR CELLS. — During the first few hours succeeding intraperitoneal injection of a sublethal dose of typhoid bacilli, the polynuclears play but an insignificant part, but in six hours there is usually a decided reaction by these cells. The peritoneal fluid begins to appear purulent, and contains vast numbers of polynuclear leucocytes. In smears made from the fluid hundreds or even thousands of these cells may be passed in review without finding any which contain bacilli. Every now and then, however, a cell may be found which is phagocytic, and, curiously enough, such cells usually contain a considerable number of bacilli, those of the immune animal distinctly more as a rule than those of the normal one. On the omentum the same phenomenon is also apparent. There are vast numbers of polynuclears, only a few of which contain bacilli. Bacilli which have been taken up by the polynuclears mostly appear intact, preserving their normal form and staining well. The phagocytic polynuclear, after taking up a certain number of bacilli, appears to remain inert until it is englobed, together with its contents, by a macrophage, after which a slow process of digestion commences.

PHENOMENA OBSERVED IN THE PERITONEAL CAVITY. — It has been mentioned that although the number of bacilli found alive in the organs of both normal and immunized animals show no appreciable differences, yet so far as regards the peritoneal cavity itself some differences are noticeable. Table XIX. gives the averages of the number of bacilli estimated to be still alive and free in the peritoneal cavity at varying intervals, column 1 showing the averages for the normal, and column 2 for the immunized animal. It must be admitted that the figures may be misleading and might not be found to hold good if a larger number of experiments were made, but conceding that they afford an indication of

the regular course of events, the figures can be used as an argument in favor of the theories of immunity already broached.

At once. — The figures for the immunized animal show a tendency to run lower than in the normal. This tendency may be accounted for by supposing that a larger number of bacilli are englobed by the macrophages and fixed on the omentum.

One and two hours. — The figures are distinctly lower for the normal than for the immune rabbit. We have seen that the blood serum is bactericidal in the case of the normal, but not bactericidal in the case of the immune rabbit. It is not known if this principle can be applied to the body fluids of the living animal, but the figures indicate that the normal peritoneal fluid can at first dispose of more bacilli left free by the macrophages than the immune fluid.

Four and six hours. — It is at this period that the differences become more marked. There is a tendency towards increase of the bacilli in the normal peritoneal fluid, whereas in the immune fluid there is progressive decrease. In the experiments so far detailed the normal rabbits were tested in the season of 1905-6, and the immunized in 1906-7, but Table XX. gives a comparative test made recently. The two rabbits were of the same size, both being inoculated and sacrificed on the same day. The numbers of bacilli in the organs are practically alike, except that in the immunized rabbit the early accumulation in the spleen, already commented upon, is apparent. But in the normal animal there are approximately one hundred and fifty millions of free bacilli in the peritoneal fluid, whereas there are only twelve millions in the fluid of the immune one. Microscopical examination of the omentum confirms the general accuracy of this observation. In the one case (normal) the bacilli have obviously been multiplying enormously, forming centers of secondary multiplication as they have been called in Part V., whereas in the other case (immune) there is little evidence of secondary increase. This question of secondary multiplication in the peritoneal cavity was discussed in Part

V., and the conclusion was reached that although the macrophages of the peritoneal fluid and omentum could take up an enormous number of bacilli, yet they could not effectively digest them, unless or until there was a good local reaction on the part of the organism, and the peritoneal cavity became filled with pus. If the reaction was delayed beyond three or four hours the bacilli would multiply enormously, not only those lying free but also those in the interior of the macrophages.

Now it has become evident in the course of these experiments that in four to six hours the immune animal on the whole shows a much more decided local reaction in the peritoneal cavity than is the case with the normal one. Nevertheless, it seems probable that this increased capacity for local reaction is not due directly to the immune condition of the animal, but indirectly, because, being immune, it does not suffer from the early liberation of the endotoxins as does the normal rabbit. The probable reasons for this difference in susceptibility have already been suggested. The chart attached to Table XX. is typical of the differences between the normal and immune rabbit in respect of temperature, except that the return towards normal occurs in both cases earlier than is usual. As a rule, the normal shows a drop followed by a rise, while the temperature of the immune rabbit remains constant or shows a rise from the start. The latter, therefore, is better equipped for an early local reaction than the former, which has first to overcome the depressing effect of the endotoxins liberated shortly after inoculation, and is not able to react locally so quickly as the animal which has felt no ill effects from inoculation.

Sixteen, twenty-four, and forty-eight hours. — Both normal and immunized rabbits by this time show good local peritoneal reaction, and are on the high road to recovery. The bacilli still alive and free in the cavity are greatly reduced in number, and there is no appreciable difference between the normal and the immunized animals.

A few remarks on opsonins. — The work of Wright and his collaborators is now so well known that no special

reference need be made to it, but the conclusions that have been reached from these studies certainly tend to support Wright's view that the opsonins are very prominent factors in immunity. The opsonic index of ordinary examinations is calculated upon the phagocytic properties of the polynuclears, but the influence of the opsonins upon the bacilli appears to make them more acceptable to the macrophages also. With this influence, however, the power of the opsonins seems to terminate, since the macrophages of the immune animal do not apparently destroy the bacilli more quickly than those of the normal one, except, perhaps, locally in the peritoneal cavity on account of local conditions. Indirectly, however, as has been suggested, the opsonins protect the immune animal, since the endotoxins liberated on disintegration of the bacilli are discharged into the plasma of cells not directly connected with the life of the organism instead of into the body fluids from which they can be absorbed into cells which are of vital importance.

CONCLUSIONS.

1. A rabbit immunized to typhoid bacilli does not dispose of a sublethal dose of living typhoid bacilli more quickly than a normal rabbit.
2. Nor does it become appreciably more resistant to the endotoxins.
3. The immunity probably resides in the increased capacity of the phagocytes, principally the macrophages, for taking up bacilli.
4. The endotoxins, therefore, on destruction of the bacilli, in the immune animal, are liberated chiefly into the cell plasma of the phagocytes, instead of directly into the body fluids as in the normal rabbit.

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TABLE I.—(At Once.)

Rabbits killed immediately after intraperitoneal injection.

	Inoculations.	Weight, Lbs.	Peritoneal Wash.	Spleen.	Liver.	Marrow.	Upper Ant. Med. Nodes. Estimated Total.	Blood per cc.	Serum.	
									Bactericidal Power.	Agglutinative Value.
1...	S.C. and I.V.	4	250,000,000	30,000	50,000	2,000	25,000,000	200,000	None.	5,000
2...	S.C. and I.V.	3½	1,000,000,000	15,000	15,000	1,500	15,000,000	20,000	None.	20,000
3...	S.C. and I.V.	4½	1,500,000,000	10,000	50,000	5,000	20,000,000	200,000	None.	10,000
4...	S.C.	4	350,000,000	2,500	15,000	2,000	6,000,000	75,000	<Normal.	10,000
5...	S.C.	4½	400,000	1,500	7,500	1,500	2,500,000	20,000	None.	5,000
6...	S.C. and I.V.	3½	300,000,000	150	500	0	350,000	10,000	None.	40,000
7...	S.C.	3½	1,200	0	0	0	250	0	Normal.	5,000

TABLE II.

Rabbits killed one hour after injection.

	Inoculations.	Weight, Lbs.	Peritoneal Wash.	Spleen.	Liver.	Marrow.	Upper Ant. Med. Nodes.	Blood per cc.	Serum.	
									Bactericidal Power.	Agglutinative Value.
1...	S.C.	4½	7,500,000	8,000	10,000	4,000	2,000,000	10,000	<Normal.	15,000
2...	S.C. and I.V.	5½	100,000,000	7,000	5,000	500	7,000,000	5,000	None.	40,000
3...	S.C. and I.V.	4	2,000,000	1,200	2,000	400	2,000,000	750	None.	2,500
4...	S.C. and I.V.	3½	30,000,000	1,000	2,000	100	500,000	1,000	None.	25,000
5...	S.C.	3	400,000	500	800	100	3,000,000	200	None.	10,000
6...	S.C. and I.V.	4½	1,200,000	450	150	20	100,000	50	None.	10,000
7...	S.C.	3½	160?	0	0	0	50	0	None.	10,000

TABLE III.

Rabbits killed two hours after injection.

	Inoculations.	Weight, Lbs.	Serum.						
			Peritoneal Wash.	Spleen.	Liver.	Marrow.	Upper Ant. Med. Nerves.	Brain per cc.	Agglutination Value.
1...	N.C. and I.V.	3½	120,000,000	5,000	11,000	1,500	25,000,000	25,000	None.
2...	S.C. and I.V.	20,000,000	700	1,500	300	300,000	150	None.
3...	N.C.	3½	25,000,000	450	5,000	350	2,000,000	2,000	None.
4...	N.C.	4½	200,000	350	450	100	1,000,000	12	Normal.
5...	N.C.	4½	150,000	50	15	5	1,000	2	Slight.
6...	N.C. and I.V.	2½	15,000,000	5	2	2	50,000	40	None.

TABLE IV.

Rabbits killed five hours after injection.

	Inoculations.	Weight, Lbs.	Serum.						
			Peritoneal Wash.	Spleen.	Liver.	Marrow.	Upper Ant. Med. Nerves.	Brain per cc.	Agglutination Value.
1...	N.C. and I.V.	2½	25,000,000	5,000	4,000	750	25,000,000	35	None.
2...	N.C.	4½	2,500,000	2,000	3,000	1,500	25,000,000	300	Slight.
3...	N.C.	4½	900,000	600	60	300	2,000,000	5	None.
4...	N.C. and I.V.	2½	25,000,000	300	250	40	20,000,000	25	Normal.
5...	N.C. and I.V.	2½	1,000,000	150	100	5	500,000	10	None.
6...	N.C.	2½	300,000	110	110	40	300,000	80	None.

TABLE V.

Rabbits killed six hours after injection.

	Inoculations.	Weight, Lbs.	Peritoneal Wash.	Spleen.	Liver.	Mar-row.	Upper Ant. Med. Nodes.	Blood per cc.	Serum.	
									Bacte-ricidal Power.	Agglu-tinative Value.
1...	S.C. and I.V.	3½	2,000,000	2,500	3,000	1,000	50,000,000	30	None.	40,000
2...	S.C.	3½	300,000	2,000	2,000	220	35,000,000	30	<Normal.	10,000
3...	S.C. and I.V.	4½	12,000,000	1,500	350	300	7,000?	50	None.	7,500
4...	S.C. and I.V.	4½	150,000	300	110	50	750,000	5	None.	30,000
5...	S.C. and I.V.	3½	16,000,000	70	220	50	1,500,000	0	None.	40,000
6...	S.C.	4½	1,200,000	40	35	150	5,000,000	4	✓Slight.	10,000

TABLE VI.

Rabbits killed sixteen hours after injection.

	Inoculations.	Weight, Lbs.	Peritoneal Wash.	Spleen.	Liver.	Mar-row.	Upper Ant. Med. Nodes.	Blood per cc.	Serum.	
									Bacte-ricidal Power.	Agglu-tinative Value.
1...	S.C.	3½	150,000	2,500	5	∞	30,000	10	> Normal.	?
2...	S.C. and I.V.	4½	450,000	2,000	65	400	25,000,000	12	None.	10,000
3...	S.C. and I.V.	4	750,000	500	45	40	10,000,000	12	None.	5,000
4...	S.C. and I.V.	4	320,000	150	20	40	2,000,000	6	None.	5,000
5...	S.C.	4	400,000	25	25	0	150,000	50	Slight.	15,000
6..	S.C.	5	100,000	20	1	12	1,000,000	0	> Normal.	10,000

TABLE III.

Rabbits killed two hours after injection.

	Inoculations.	Weight, Lbs.	Peritoneal Wash.	Spleen.	Liver.	Mar-row.	Upper Ant. Med. Nodes.	Blood per cc.	Serum.	
									Bacte-ricidal Power.	Agglu-tinative Value.
1...	S.C. and I.V.	3½	120,000,000	5,000	10,000	1,500	35,000,000	25,000	None.	40,000
2...	S.C. and I.V.	20,000,000	700	1,500	300	300,000	750	None.	10,000
3...	S.C.	3½	25,000,000	650	3,000	250	2,000,000	2,000	None.	10,000
4...	S.C.	4½	200,000	350	450	200	1,000,000	12	Normal.	10,000
5...	S.C.	4½	150,000	50	15	8	12,000	0	Slight.	10,000
6...	S.C. and I.V.	2½	15,000,000	6	0	0	150,000	40	None.	7,500

TABLE IV.

Rabbits killed four hours after injection.

	Inoculations.	Weight, Lbs.	Peritoneal Wash.	Spleen.	Liver.	Mar-row.	Upper Ant. Med. Nodes.	Blood per cc.	Serum.	
									Bacte-ricidal Power.	Agglu-tinative Value.
1...	S.C. and I.V.	3½	25,000,000	6,000	4,000	750	35,000,000	350	None.	25,000
2...	S.C.	4½	2,500,000	2,000	3,000	2,500	35,000,000	300	Slight.	50,000
3...	S.C.	4½	800,000	600	60	300	2,000,000	50	None.	5,000
4...	S.C. and I.V.	3½	25,000,000	300	250	40	20,000,000	25	<Normal.	10,000
5...	S.C. and I.V.	3½	1,000,000	150	100	5	500,000	10	None.	40,000
6...	S.C.	3½	300,000	110	110	40	300,000	80	None.	5,000

TABLE V.

Rabbits killed six hours after injection.

	Inoculations.	Weight, Lbs.	Peritoneal Wash.	Spleen.	Liver.	Mar-row.	Upper Ant. Med. Nodes.	Blood per cc.	Serum.	
									Bactericidal Power.	Agglutinative Value.
1...	S.C. and I.V.	3½	2,000,000	2,500	3,000	1,000	50,000,000	30	None.	40,000
2...	S.C.	3½	300,000	2,000	2,000	220	35,000,000	30	< Normal.	10,000
3...	S.C. and I.V.	4½	12,000,000	1,500	350	300	7,000?	50	None.	7,500
4...	S.C. and I.V.	4½	150,000	300	110	50	750,000	5	None.	30,000
5...	S.C. and I.V.	3½	16,000,000	70	220	50	1,500,000	0	None.	40,000
6...	S.C.	4½	1,200,000	40	35	150	5,000,000	4	✓ Slight.	10,000

TABLE VI.

Rabbits killed sixteen hours after injection.

	Inoculations.	Weight, Lbs.	Peritoneal Wash.	Spleen.	Liver.	Mar-row.	Upper Ant. Med. Nodes.	Blood per cc.	Serum.	
									Bactericidal Power.	Agglutinative Value.
1...	S.C.	3½	150,000	2,500	5	∞	30,000	10	> Normal.	?
2...	S.C. and I.V.	4½	450,000	2,000	65	400	25,000,000	12	None.	10,000
3...	S.C. and I.V.	4	750,000	500	45	40	10,000,000	12	None.	5,000
4...	S.C. and I.V.	4	320,000	150	20	40	2,000,000	6	None.	5,000
5...	S.C.	4	400,000	25	25	0	150,000	50	Slight.	15,000
6...	S.C.	5	100,000	20	1	12	1,000,000	0	> Normal.	10,000

TABLE VII.

Rabbits killed twenty-four hours after injection.

	Inoculations.	Weight, Lbs.	Peritoneal Wash.	Spleen.	Liver.	Marrow.	Upper Ant. Med. Nodes.	Blood 1 cc.	Serum.	
									Bactericidal Power.	Agglutinative Value.
1...	S.C. and I.V.	5	80,000	4,000	100	60	1,500,000	1	None.	25,000
2...	S.C. and I.V.	4	50,000	500	5	0	1,500,000	0	None.	5,000
3...	S.C.	4	60,000	160	4	10	100,000	3	Normal.	5,000
4...	S.C.	4	100,000	150	8	50	500,000	14	Normal.	2,000
5...	S.C. and I.V.	4	150,000	30	0	0	300,000	0	None.	50,000
6...	S.C.	3½	360,000	8	2	0	20,000	0	<Normal.	10,000

TABLE VIII.

Rabbits killed forty-eight hours after injection.

	Inoculations.	Weight, Lbs.	Peritoneal Wash.	Spleen.	Liver.	Marrow.	Upper Ant. Med. Nodes.	Blood per cc.	Serum.	
									Bactericidal Power.	Agglutinative Value.
1...	S.C. and I.V.	4½	3,000	400	0	0	50,000	0	None.	10,000
2...	S.C. and I.V.	4	15,000	250	2	0	50,000	0	None.	5,000
3...	S.C.	5	0	150	75	10	100,000	0	>Normal.	5,000
4...	S.C. and I.V.	4½	0	20	0	0	30,000	0	None.	10,000
5...	S.C.	4	20,000	6	0	0	10,000	0	Normal.	5,000
6...	S.C.	4½	2,000	2	2	0	2,000,000	100	Normal.	5,000

TABLE IX. (Spleen.)

Colonies of typhoid bacilli on spleen plates. Six rabbits at each interval.

	At once.	1 hour.	2 hours.	4 hours.	6 hours.	16 hours.	24 hours.	48 hours.
1	30,000	8,000	5,000	6,000	2,500	2,500	4,000	400
2	15,000	7,000	700	2,000	2,000	2,000	500	250
3	10,000	1,200	650	600	1,500	500	160	150
4	2,500	1,000	350	300	300	150	150	20
5	1,500	500	50	150	70	25	30	6
6	150	450	6	110	40	20	8	2

TABLE X. (Liver.)

Colonies of typhoid bacilli on liver plates. Six rabbits at each interval.

	At once.	1 hour.	2 hours.	4 hours.	6 hours.	16 hours.	24 hours.	48 hours.
1	50,000	10,000	10,000	4,000	3,000	65	100	75
2	50,000	5,000	3,000	3,000	2,000	45	8	2
3	15,000	2,000	1,500	250	350	25	5	2
4	15,000	2,000	450	110	220	20	4	0
5	7,500	800	15	100	110	5	2	0
6	500	150	0	60	35	1	0	0

TABLE XI. (Blood.)

Typhoid bacilli per cubic centimeter of the blood. Six rabbits at each interval.

	At once.	1 hour.	2 hours.	4 hours.	6 hours.	16 hours.	24 hours.	48 hours.
1	200,000	10,000	25,000	2,500	50	50	14	100
2	200,000	5,000	2,000	750	30	12	3	0
3	75,000	1,000	750	300	30	12	1	0
4	20,000	750	40	40	5	10	0	0
5	20,000	200	12	40	4	6	0	0
6	10,000	50	0	5	0	0	0	0

TABLE XII.

Upper anterior mediastinal lymph nodes.

	At once.	1 hour.	2 hours.	4 hours.	6 hours.	16 hours.	24 hours.	48 hours.
1	25,000,000	7,000,000	35,000,000	35,000,000	50,000,000	25,000,000	1,500,000	2,000,000
2	20,000,000	3,000,000	2,000,000	35,000,000	35,000,000	10,000,000	1,500,000	100,000
3	15,000,000	2,000,000	1,000,000	20,000,000	5,000,000	2,000,000	500,000	50,000
4	6,000,000	2,000,000	300,000	2,000,000	1,500,000	1,000,000	300,000	50,000
5	2,500,000	500,000	50,000	500,000	750,000	150,000	100,000	30,000
6	350,000	100,000	12,000	300,000	? 7,000	30,000	20,000	10,000

TABLE XIII. Peritoneal Wash.
Bacteria alive and free in peritoneal cavity.

	At once.	1 hour.	2 hours.	4 hours.	6 hours.	16 hours.	24 hours.	48 hours.
1. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100	150,000,000	120,000,000	25,000,000	25,000,000	750,000	350,000	20,000	
2. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100	70,000,000	25,000,000	25,000,000	12,000,000	450,000	150,000	15,000	
3. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100	7,500,000	20,000,000	2,500,000	2,000,000	400,000	100,000	3,000	
4. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100	2,000,000	15,000,000	1,000,000	1,200,000	320,000	80,000	2,000	
5. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100	1,200,000	200,000	800,000	300,000	150,000	60,000	0	
6. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100	400,000	15,000	300,000	150,000	100,000	50,000	0	

TABLE XIV.
Averages of middle four animals from Tables IX. to XIII.

	Blood per cc. Table XI.	Spleen. Table IX.	Liver. Table X.	Ant. Med. Nodes. Table XII.	Peritoneal Wash. Table XIII.
1... At once.	80,000	7,500	20,000	10,000,000	500,000,000
2... 1 hour.	1,750	2,500	2,500	2,000,000	12,000,000
3... 2 hours.	700	450	1,250	1,000,000	15,000,000
4... 4 hours.	300	750	850	14,000,000	7,500,000
5... 6 hours.	20	970	675	10,000,000	4,000,000
6... 16 hours.	10	700	25	3,250,000	350,000
7... 24 hours.	1	200	5	600,000	100,000
8... 48 hours.	0	100	1	60,000	5,000

TABLE XV.

Comparative normal and immune. The figures for the normal rabbits are taken from Part II. of this work. Those for the immune rabbits from Tables I. to VII.

	AT ONCE.				ONE HOUR.			
	Spleen.		Liver.		Spleen.		Liver.	
	Normal.	Immune.	Normal.	Immune.	Normal.	Immune.	Normal.	Immune.
1.....	3,500	30,000	25,000	50,000	1,500	8,000	3,000	10,000
2.....	3,000	15,000	25,000	50,000	500	7,000	2,500	5,000
3.....	2,000	10,000	15,000	15,000	425	1,200	2,000	2,000
4.....	1,000	2,500	7,000	15,000	20	1,000	500	2,000
5.....	0	1,500	0	7,500	?	500	225	800
6.	0	150	0	500	0	450	0	150

TABLE XVI.

Comparative normal and immune.

	TWO HOURS.				FOUR HOURS.			
	Spleen.		Liver.		Spleen.		Liver.	
	Normal.	Immune.	Normal.	Immune.	Normal.	Immune.	Normal.	Immune.
1.....	525	5,000	1,500	10,000	3,000	6,000	20,000	4,000
2.....	200	700	1,000	3,000	2,000	2,000	5,000	3,000
3.....	50	650	100	1,500	150	600	1,000	250
4.....	15	350	100	450	25	300	150	110
5.....	15	50	25	15	10	150	100	100
6.....	0	6	0	0	0	110	0	60

TABLE XVII.
Comparative normal and immune.

	Six Hours.				Sixteen Hours.			
	Spleen.		Liver.		Spleen.		Liver.	
	Normal.	Immune.	Normal.	Immune.	Normal.	Immune.	Normal.	Immune.
1....	4,000	2,500	25,000	3,000	1,500	2,500	600	65
2....	1,000	2,000	15,000	2,000	1,200	2,000	150	45
3....	450	1,500	6,000	350	100	500	25	25
4....	200	300	1,000	220	30	150	15	20
5....	?	70	75	110	25	25	3	5
6....	0	40	0	35	0	20	0	1

TABLE XVIII.
Comparative normal and immune.

	Twenty-four Hours.				Forty-eight Hours.			
	Spleen.		Liver.		Spleen.		Liver.	
	Normal.	Immune.	Normal.	Immune.	Normal.	Immune.	Normal.	Immune.
1.....	800	4,000	4,000	100	650	400	3	75
2.....	400	500	15	8	200	250	2	2
3.....	150	160	12	5	20	150	1	2
4.....	30	150	3	4	10	20	0	0
5.....	15	30	0	2	0	6	0	0
6.....	0	8	0	0	0	2	0	0

TABLE XIX. (Peritoneal Wash.)

Comparison between normal and immunized rabbits. The estimated number of bacilli left alive in the peritoneal cavity. Averages of middle four rabbits in each table. The figures for the normal rabbits are taken from tables in Part II. of this work.

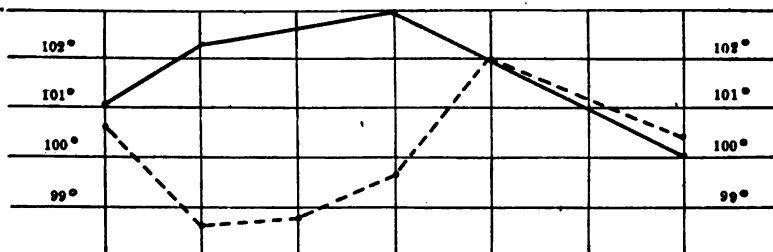
		Normal. Tables in Part II.	Immune. Table XIII.			Normal. Tables in Part II.	Immune. Table XIII.
1 ...	At once.	1,500,000,000	500,000,000	5...	6 hours.	35,000,000	4,000,000
2 ...	1 hour.	1,750,000	12,000,000	6...	16 hours.	400,000	350,000
3 ...	2 hours.	2,250,000	15,000,000	7...	24 hours.	400,000	100,000
4 ...	4 hours.	35,000,000	7,500,000	8...	48 hours.	170,000	5,000

TABLE XX.

Comparative normal and immune. Six hours after inoculation with temperature chart attached.

	Peritoneal Wash.	Spleen.	Liver.	Marrow.
Normal	150,000,000	500	1,500	80
Immune	12,000,000	1,500	350	300

Temperature chart: normal ---. Immune —.



SOLID TERATOMATA OF THE MEDIASTINUM.*

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In 1902 under the title "Dermoid Cysts and Teratomata of the Anterior Mediastinum" ¹ I published a case of simple dermoid cyst of the anterior mediastinum and analyzed the reported cases of this condition. The cases were classified in that paper as follows: "1st, those of slight complexity, which are essentially dermoid cysts of ectodermal origin; 2d, those of great complexity which contain derivatives from all three germ layers with the formation of rudimentary organs and which may be regarded as teratomata; 3d, tumors of the first or second class which in some part of their structure are malignant and form metastases in other organs." At that time I could collect forty cases, and of these two were grouped under class second, "those of great complexity—which may be regarded as teratomata." In Ekehorn's ² case, besides a dermoid cyst, there was a bone resembling the superior maxilla, ganglia suggesting spinal ganglia arranged between bits of bone, and structures somewhat resembling fetal lung and intestine. Virchow's case ³ showed much young striated muscle, and tissue which suggested fetal lung in addition to areas of a sarcomatous and carcinomatous nature.

Since 1902 I have seen specimens from three additional cases, one simple dermoid cyst, and two solid teratomata. The latter I report since they belong to the rarer group (2) of my classification and show a large amount of neuroglia tissue. Only one other case, that of Bull, ⁴ similar in this latter regard, has been published in a total of sixty cases which I was able to analyze recently. ⁵ Of these sixty cases, seven belonged to the teratoma group, as follows: Cases of Ekehorn, ⁶ Virchow, ⁷ Bull, ⁸ Fofanow, ⁹ Warthin ¹⁰ and the author's two cases. The case of Bull and the author's two

* Received for publication April 10, 1907.

cases form a group presenting quite similar pathological characteristics.

Case I.* was a boy (a patient of the late Dr. J. M. Sheahan, of Quincy, Mass.), age nineteen, who died with a history of symptoms, referable to the mediastinal tumor, of three months' duration. The tumor weighed four thousand seven hundred and fifty grams, and occupied nearly the whole of the right half of the thorax. The lung, presented as a flattened lobe, crowded into the postero-lateral portion of the upper part of the pleural cavity. The right bronchus was embedded in the tumor. The heart was crowded over to the left in an almost horizontal position and the left lung was in consequence somewhat compressed. There was no evidence of metastasis of the tumor into any of the organs of the body. The tumor was lobulated, firm, grayish to pink in color and contained many small cysts filled with colloid material and a few larger cysts several centimeters in diameter.

Numerous pieces of tissue were taken from various parts of the tumor for histological examination. These were hardened in formalin, mordanted by the picric acid ammonium bichromate method, embedded in paraffin and sectioned. The sections were stained with Mallory's phosphotungstic acid hematoxylin with and without differentiation in ferric chloride.†

The tumor consists of many cysts of varying shape. Most of them are round or oval, one-half to three millimeters in diameter. Some appear as slits or clefts; others have an irregular contour; a few show slight papillary ingrowths. There are a few cysts considerably larger, up to several centimeters in diameter. Some of the larger cysts have undoubtedly resulted from the coalescence of adjacent cysts as the intervening septa thinned with the increase in size of the cysts.

Many of the cysts contain a hyaline or granular coagulum

* Both cases are reported in the chapter in the *System of Medicine* above referred to, clinically at slight length, pathologically very briefly.

† The technical methods employed here may be found described in Mallory and Wright's *Pathological Technique*, published by W. B. Saunders & Co., Philadelphia, 1904.

enclosing desquamated cells. Others are empty as they appear in section. A number of them contain desquamated cornified epithelium. The total area of cyst in a square centimeter varies much in different parts of the tumor. Few blocks of tissue one centimeter square are free of cysts. The average number is five to ten. In some parts they are crowded together so that merely thin septa of stroma intervene.

Many varieties of epithelium line these cysts. High cylindrical ciliated epithelium is common, usually in stratified form. The cilia are relatively long and show distinctly basal dots below the point of their insertion in the cell. Some cysts are lined by a single layer of cylindrical or cuboidal ciliated epithelium. Simple unciliated epithelium of the same types occurs rather more frequently. Occasionally this is of the mucous type with goblet cells. Simple squamous and transitional epithelium is frequently found, and still more common is epithelium of epidermal type with prickle cells, keratohyaline granules, and distinct stratum corneum. In connection with this last type, in a few places, hair follicles, sebaceous glands, and sweat ducts are seen. Very rarely is seen a cyst lined by low cuboidal epithelial cells containing coarse rod-shaped brownish pigment granules similar to those occurring in the retina. In the connective tissue adjacent to these cysts are found larger cells filled with similar pigment. In both, the small round nucleus is almost obscured by the pigment. In close association with this pigmented epithelium in two places is a cyst of irregular contour containing numerous small vascular papillary ingrowths covered by low cylindrical or cuboidal epithelium. These epithelial cells have in their outer end a row of small dots which stain in the same way as do neuroglia fibrils and in this respect these cells resemble ependymal epithelium. The vascular stalks thus covered bear some resemblance to the choroidal plexus of the cerebral ventricles. The presence of these dots, however, is not proof of the ependymal nature of epithelial cells as has already been pointed out by me¹¹ and by Verhoeff,¹² who has shown the frequent occurrence of a

pair of dots with similar staining reaction in a variety of lining epithelium. Moreover, both these dots and the limiting membrane described by Verhoeff¹³ is seen in much of the epithelium lining the cysts of this tumor.

In addition to cysts, numerous groups of small glands of the branching tubular type lined by high and low cylindrical mucous epithelium occur. None of this epithelium is ciliated. About these cysts there are no definite layers of smooth muscle or connective tissue, but the glands are scattered irregularly in a loose connective tissue stroma. Numerous small irregular solid masses of epithelium of epidermal type are found, sometimes with central concentric cornified cell masses forming epithelial pearls.

In one section is a group of epithelial cells somewhat resembling liver tissue. The cells are roughly cuboidal in shape, averaging fourteen microns in diameter. The cytoplasm is dense, finely granular and deeply staining. Some cells contain small fat vacuoles. Nuclei are round or oval, vesicular with one large rounded chromatin mass. The cells are arranged in irregular columns two cells thick. In many places between the cells is seen a narrow lumen, whose margin stains darkly with phosphotungstic acid hematoxylin. This lumen branches and is distributed between the cells in a way resembling biliary canaliculi, which likewise have this staining reaction. Cell columns are related to each other in a manner not unlike columns of liver cells and between them run capillaries, but there is no vascular space analogous to sinusoids. There is no pigment resembling biliary pigment.

Besides cysts and glands lined by epithelium of these various developed types there are a few small solid masses and glands composed of slightly differentiated epithelium of embryonal type.

Usually a cyst is lined throughout by the same type of epithelium, but many show two or three different types with transitions from one type to another, as we find normally where esophagus and stomach join or where vaginal and uterine mucosa fuse.

The stroma between the cysts is as varied as the epithelium lining them. Fibrous tissue predominates and ranges in type from dense tissue with coarse bundles of wavy parallel fibers and a few compressed elongated cells to loose cellular tissue composed of numerous large cells with round or oval vesicular nuclei and numerous protoplasmic processes lying in the interstices of fine interlacing fibrils. In places the fibrous tissue is evidently edematous but no true myxomatous tissue is found.

Large areas of adipose tissue are common. Much of this is of the type composed of large fat droplets surrounded by a thin cytoplasmic zone with a crescentic nucleus crowded to one side. Many of the nuclei contain fat droplets. In other places the adipose tissue is of the embryonic type, a loose connective tissue containing cells partially filled by a single fat droplet or several smaller ones.

Unstriated muscle is present in almost all parts of the tumor as scattered narrow bundles running in different directions. Small groups of striated muscle fibers occur in one or two places. Small masses of hyaline cartilage are frequently seen and a few of these show bone formation. In the spaces between bone trabeculae no evidence of marrow formation is found, though there are large blood sinuses. Both bone and cartilage occur also in an only partially differentiated state.

Here and there in the tumor are small masses of neuroglia. These bear no special relation to cysts. The neuroglia never forms a very large part of a section and in numerous sections does not occur. The masses vary much in shape; a few are round or oval; most are extremely irregular. At the margin usually there is quite a sharp separation between neuroglia and fibrous tissue, but in places there is an intimate intermingling of neuroglia and fibrous tissue fibrils. In such places neuroglia fibrils may extend for some distance into fibrous tissue unaccompanied by any demonstrable neuroglia cells.

Neuroglia masses vary much in the character and abundance of fibrils. Some masses are made up of rather widely

separated, very fine, interlacing fibrils approaching in structure the normal neuroglia framework of the human central nervous system. Much more common are rather coarse fibrils, tending to a parallel course in many instances, interlacing in others. A few areas are made up of very coarse fibrils. Often fine and coarse fibrils are mingled in varying proportions. In some cases the structure is loose and there are many spaces, in others there is a very dense feltwork of fibrils. Most of the fibrils run a straight or slightly wavy course. Here and there are zigzag fibrils or fibrils with recurved ends as if an elastic fibril had been suddenly relieved of tension. Individual fibrils are long, of quite uniform contour, and do not branch.

Neuroglia cells vary much both in number and size. In some foci they are very sparse, in others numerous. There are all gradations between a cell with small round or oval deeply-staining vesicular nucleus and very little cytoplasm and a large cell with palely-staining vesicular nucleus and large cytoplasmic body. Many of the larger cells possess several long processes that can be traced for some distance among the fibrils. In places the cells are roughly rectangular and closely crowded together. Very commonly the nuclei are eccentrically placed. In many cells can be seen one or two small granules, staining like the neuroglia fibrils, often surrounded by a paler zone. The cytoplasm of the neuroglia cells stains fairly deeply and has a homogeneous ground-glass appearance. A few cells are very large, measuring thirty to forty microns in diameter.

Besides these forms of differentiated stroma there are a few small areas of embryonal mesenchyma, as yet undifferentiated into any definite form of connective tissue.

Ganglion cells are found in a few sections in small groups or somewhat scattered in the tissue. The groups of cells resemble spinal ganglia. The cells have large vesicular nuclei, distinct nucleoli and cell bodies varying in size from twenty to forty-four microns. Nissl bodies occur though the tissue was not preserved in a way suitable for a very satisfactory demonstration of them. Many of the cells have

a distinct capsule of cells with flattened nuclei. Some of the cells show a distinct process which can be traced a short distance. A single isolated large ganglion cell, identical with these described here but free of capsule, was found in one mass of tissue consisting of a dense network of rather coarse neuroglia fibrils and numerous neuroglia cells.

The tumor is moderately vascular. The vessel walls are thin for the most part. Nowhere in the tumor is there evidence of active cell multiplication.

Case II. (a patient seen in consultation with Dr. W. A. Griffin, of Sharon, Mass.) was a boy of seventeen, who was operated on about one year after the onset of symptoms referable to a mediastinal tumor, and a large mass of tissue was removed from the right thorax. Complete removal of the tumor was impossible and, after temporary improvement, there was regrowth of the tumor and gradual decline in the patient's condition until he died two and one-half months after the operation.* At operation there was found a fairly large cyst cavity containing fluid, hairs, and sebaceous material. Many solid masses projected into this or were connected with its wall. The tissue removed at operation consisted of two masses measuring $16 \times 8 \times 7$ centimeters, and $8 \times 8 \times 6$ centimeters, and two small masses. The larger were devoid of epithelial covering, gray flecked with areas of pink or red, tough and elastic. The cut surface showed many small cysts filled with a viscid, glairy, colorless fluid. Between the cysts lay a grayish, semi-translucent, edematous tissue, fairly homogeneous in appearance. One of the smaller masses was a teat-like process covered by epidermis from which grew hairs.

Numerous portions of this tumor were hardened in Zenker's fluid and ten per cent formalin, embedded in paraffin and sectioned. The Zenker fixed material, in particular, was studied and this was stained with the eosin methylene blue and the phosphotungstic acid hematoxylin methods.

Sections of the tumor contain many cysts from one-half to

* For a more detailed clinical history see Griffin, Boston Medical and Surgical Journal, 1907, clvi, 9.

eight millimeters in diameter. The smaller cysts are more numerous. The distribution of cysts is not so uniform as in the preceding case. Some sections contain only a few of the smaller cysts; others are almost entirely made up of cysts separated by only the narrowest zone of stroma. The cysts vary in shape, round, oval or irregular. Many contain hyaline or granular coagulum with desquamated epithelial cells. Rarely a cyst is filled with desquamated cornified epithelium.

Many of the cysts are lined by high cylindrical epithelium, sometimes as a single layer of cells, more often as several layers in the form of a stratified cylindrical epithelium. Some of this epithelium is unciliated, some ciliated. The latter is relatively less common than in the first tumor. Much of the epithelium is of the mucous type with goblet cells. A number of cysts are lined by high cylindrical epithelium with a clear cytoplasm as if most of the cell content had been dissolved out. In this latter form of epithelium the nuclei of the cells at times occupy uniformly the basal end of the cell, at others the distal end. All of these varieties of cells appear also in the form of low cuboidal epithelium, but this is not so common.

A few cysts are lined by epithelium of the transitional type, by simple squamous epithelium or by squamous epithelium of the epidermal type. Here and there a cyst shows numerous branching papillary ingrowths covered by low cuboidal epithelium. These cells, however, have only a very superficial resemblance to ependymal epithelium. There are also numerous tubular glands lined by low or high columnar epithelium, not ciliated. Many cysts show several types of epithelium lining a single cyst.

Many of the cysts in this tumor stand in close relation to neuroglia masses in that their lining epithelium lies in immediate juxtaposition to the neuroglia. In most cases this epithelium resembles that lining the spinal canal of the fetus and consists of a layer of cells with round or oval vesicular nuclei and cell bodies whose outlines for the most part cannot be made out. The nuclei have a narrow clear zone

between them and the cyst cavity, in the outer edge of which are small dots staining dark blue with phosphotungstic acid hematoxylin. When cut obliquely an indistinct limiting membrane with polygonal meshes can be made out, in the center of each of which lies a pair of these dots. The nuclei lie close together, several layers deep, and there is no line of demarcation between the lining epithelium and underlying neuroglia. The latter is of the undifferentiated type and its cells have nuclei similar to those in the cells lining the cyst. These cells are most numerous in that portion of the neuroglia near to the cavity. In all of these respects there is a close relation to the ependyma of the fetus. There are also cysts lined by flattened cuboidal epithelium whose individual cells are more sharply demarcated to form a distinct layer lying on neuroglia tissue but quite sharply separated from it. These latter cells have dots in their outer end arranged in a row and more than two to each cell. The underlying neuroglia is of a more adult type with well-developed fibrils. This type, however, is less common than the more undifferentiated one.

Besides cysts and glands lined by differentiated epithelium there are many small cavities lined by epithelium of an undifferentiated type. Cell outlines are very indistinct and usually there can be made out only a clear zone of cytoplasm about the cavity and deeper down several layers of closely-packed deeply-staining round or oval vesicular nuclei. This layer is in no wise demarcated from an underlying cellular stroma. Neither epithelium nor stroma is sufficiently differentiated for its nature to be determined.

In many of the sections there are small areas made up of columns and nests of epithelial cells. These are two to four cells thick and between them run small blood vessels and a slight amount of connective-tissue stroma. These cells are rectangular or polygonal with a finely granular cytoplasm. The nuclei are vesicular and many contain a distinct nucleolus-like chromatin mass. Many of the cells contain fat droplets. Between these cells frequently is seen a narrow lumen, staining in the phosphotungstic acid

hematoxylin preparations in the same way as do bile canaliculi in liver tissue. These cells are similar to those described in the first tumor as resembling liver cells.

The stroma of this tumor consists of all gradations between embryonal undeveloped mesenchyma and differentiated tissue. There is cellular and cell-poor fibrous tissue, cartilage, rarely bone, smooth muscle, adipose and neuroglia tissue. The more cellular less well differentiated tissues occur in the more solid portions of the tumor, while the more adult types occur in those portions in which the cysts are closely crowded together.

Neuroglia tissue occurs in all gradations between the cellular type with a rare fine fibril and the older type with an interlacing meshwork of coarser fibrils. The very coarse fibrils as described in tumor I. do not occur and all of the neuroglia is quite cellular. The cells themselves are the smaller types and rarely show more than a slight rim of cytoplasm about the nucleus, though the latter may be quite large. In only a few cells can a distinct dot or pair of dots staining like the neuroglia fibrils be made out. Much of the neuroglia, especially the less differentiated types, is in close relation to cysts as already described. However, there are scattered irregular masses in the fibrous tissue as in the first tumor. Relatively in this tumor neuroglia is very much more abundant than in the first and in some parts constitutes the bulk of the tumor.

In one section there is a large group of typical ganglion cells with Nissl granules, large nucleus, distinct nucleolus, and a capsule of flattened cells having palely staining vesicular nuclei. The tumor is moderately vascular. In the more cellular portions both epithelial and stroma cells show numerous mitoses but none of atypical form as is common in malignant tumors. These parts of the tumor are evidently growing quite rapidly and in this respect resemble tissues of a developing embryo. Aside from this there is nothing in the tumor to suggest malignancy. However, in the absence of an autopsy, metastasis cannot be excluded,

and metastasis did occur in the case reported by Bull, which tumor in many particulars resembled this one.

In both tumors a few areas of necrosis with fibrin and scant leucocytes occur. Here and there a moderate infiltration with plasma and lymphoid cells is found.

The case of Bull (*loc. cit.*) was a boy of seventeen, who two years before his death began to have shortness of breath on exertion. This gradually increased and was accompanied by cough and sparse expectoration. The sputum at times was blood streaked. For some six months before his death there were severe symptoms of mediastinal obstruction. At autopsy a large tumor (15 × 15 centimeters) was found occupying the upper mediastinum and extending out into the left thoracic cavity. There was also involvement of the sternum and adjacent ribs with metastases in the left supra- and infra-clavicular and axillary regions, in the lungs, and the liver. The tumor mass contained great numbers of cysts which, on microscopic examination, were found to be lined with a variety of epithelium. The stroma consisted of fibrous connective tissue, smooth muscle, fat, cartilage, bone, and neuroglia. Some ganglion cells were found and some cysts had a pigmented epithelium similar to the pigmented epithelium of the eye. In places there were areas of irregular epithelial proliferation. In histological structure there was a close similarity to the tumor reported above (Case 2). The striking difference lies in the marked malignancy of Bull's case. The metastases were mainly of epithelial structure and resembled closely a malignant adenoma.

These three cases present a number of similar characteristics and constitute a fairly definite group of mediastinal teratomata. All occurred in young adults (19, 17, and 17 years, respectively), and made their presence manifest by symptoms developing in the period following shortly after puberty. Duration of symptoms was relatively short as compared with most teratoid tumors of this region (3 months, 1 year, and 2 years, respectively.) The tumor grew from the mediastinum, but largely occupied the pleural cavity in each case. The bulk of the tumors consisted of a solid mass of

tissue containing many small cysts. The cysts were lined by a variety of epithelium, and between the cysts was a stroma composed of unstriated muscle, fat, cartilage, bone, fibrous tissue, and neuroglia; the tumors thus made up are of great complexity of structure and resemble in this aspect tumors which are far more common in ovary and testicle, and for which the name *embryomata* has been suggested. One case, that of Bull, was malignant in the sense of metastasizing, the question of metastasis in another is uncertain as no autopsy was permitted; in one, numerous mitoses indicated rapid growth and in many places the tissue of each was of a fetal type; consequently, the group is to be regarded as malignant rather than benign. As to etiology nothing can be said. However, such complex tumors occurring in the mediastinum do not justify the assumption for ovarian and testicular teratomata of a special origin from the sexual cells of these organs, an assumption that is frequently made, but a satisfactory etiological explanation for the one should explain the other. However, it is not the province of this paper to discuss the hypotheses which have been advanced to explain the origin of teratomata.

[This work was done in the Sears Laboratory of Pathology of the Harvard Medical School, to the Director of which I take pleasure in expressing my thanks for the facilities there furnished me.]

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HEMOLYTIC PROPERTIES OF ORGAN AND TUMOR EXTRACTS.*

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PREFACE.

NOTE.

In an article which appeared after this had gone to press (Ueber ein Komplexes Hæmolysin der Bauchspeicheldrüse, by Dr. U. Friedemann, in the Deutsche Medizinische Wochenschrift, No. 15, 1907), there is a very striking confirmation of the findings in the present research. Friedemann found that an extract of beef pancreas, when activated by guinea-pig serum (probably a lecithin component), was hemolytic for the red cells of the same species. The extract played the part of an amboceptor, and could be exhausted of its active substance; the guinea-pig serum acted like complement. In the presence of the serum of the ox, however, the reaction fails to occur.

It is evident that, substituting an alien serum for red blood cell derivative (R.B.C.D.) the results are identical. It is, of course, possible that serum contains the same lecithin-like substance as does R.B.C.D.

See also, Proc. Soc. Ex. Med. and Biology, IV., Dec. 19, 1906.

the peritoneal cavity, and this led to the examination of those organs in which, supposedly, the macrophages have their origin. "The lymphatic glands of the mesentery, the

* Received for publication April 9, 1907.

glandular portion of the omentum, and the spleen, the three preëminently macrophagic organs," were therefore removed from guinea-pigs, extracted in salt solution, and their digestive effect tested upon the red blood corpuscles of the goose. It was found that the extracts brought about solution of the hemoglobin; and, further, that they lost their activity when subjected to a temperature of 56° C. for three-quarters of an hour. This investigation was then taken up in greater detail by Tarassevitch¹⁷ of the Pasteur Institute, who confirmed and amplified the results of Metchnikoff. He found that the macrophagic organs, and the pancreas, and, in a less degree, the submaxillary glands of guinea-pigs, rabbits, and dogs, possess a hemolytic action upon the red cells of birds, and of certain mammals. Moreover, the digestive ferment of these organs in solution is, as stated by Metchnikoff, thermolabile at 56° C.; the entire extract, which is, in reality, partly a solution of organ ferments, and partly an emulsion of organ particles, is thermolabile at slightly higher temperatures (58.5° – 62° C.), owing to the relative inaccessibility of the solid particles to heat. Similar results were obtained by Klein⁴ and by Shibayama.¹⁵

The Frankfort Institute, under the direction of Ehrlich, now became interested in a line of research which promised to disclose the origin of the "complements" of the serum, and in 1902 Morgenroth and Korschun⁵ published an elaborate paper on the hemolytic properties of organ extracts. They reached the conclusion that the organ extracts do not act in a manner at all comparable to that of the hemolysins of the serum, with which it had been the object of the French school to identify them. This differentiation they base on the fact that the hemolytic substances of the organ extracts are, in the first place, coctostable, and not, as had been asserted by previous investigators, destroyed by a temperature below 62.5° C. These hemolysins are, moreover, soluble in alcohol, are not complex, in the sense of an amboceptor-complement reaction, and are not able to excite the production of anti-bodies. In spite of these facts, they regard the selective action of the extracts on certain species

of blood cells, and their inactivity towards others, as indicative of a "certain specificity, which is of special biological interest." They further draw attention to the fact that the organ extracts are able to dissolve the blood cells of the same species, and even of the same individual from which they are derived.

At this point, investigation into the hemolytic properties of organ extracts ceased to attract further attention, and interest shifted to the action of the extracts made from tumors. It had always been common knowledge that a characteristic of malignant tumors was to produce cachexia and secondary anemia in their victims. Marchand⁸ interpreted the necroses, thromboses, hemorrhages, and round cell infiltration of these tumors as evidences of a toxic influence. He went even further; he suggested that the disturbance of tissue equilibrium, to which Weigert had attributed the rapid advance of tumor cells, was due to the destructive influence of this toxic substance on the surrounding tissues. It has frequently been suggested that the anemia of tumors is probably due to the action of some toxin elaborated by the tumor, and diffused into the circulating blood. The urobilinuria, which accompanies certain of the malignant tumors, and the peculiar resistance of the red blood cells to anistonic solutions in malignant cachexia, have been cited in evidence of the existence of some such toxic influence on the blood cells. During the last ten years the problem of demonstrating the existence of such toxins, and of determining their manner of action, has been approached by a variety of methods. The study of the toxicity of the urine, of the autolytic products, and of the ferments of tumors has not, however, tended to explain their hemolytic activity. It is only since the year 1902 that this problem has been approached by means of what might be described as a direct method, *i.e.*, the study of the effect of the extracts of the tumors upon emulsions of the red cells in vitro. Up to the present time, three researches devoted to this method of investigation have appeared, and, in addition, a number of isolated observations are scattered through the literature.

The hemolytic effect of tumors was first tested in 1902, by U. G. Panzacchi. He made use of a cancer of the rectum, an ulcerated cancer of the breast, a glandular metastasis of a cancer of the breast, an adenoma of the breast, and a sarcoma of the breast. These tumors were tested upon the blood cells of various mammals. All of them proved to be hemolytic. In one case only was the extract inactivated by exposure to 55° C. for one-half hour. In 1903 Micheli and Donati¹⁹ examined ten cancers, four sarcomas, and one endothelioma. Of these seven were inactive, five hemolysed all the blood tested, and three hemolysed some, but not all. None of these extracts were thermostable, or soluble in alcohol.

In 1904, Kullmann,⁶ in Germany, published a very long paper on the same subject. He prepared extracts of freshly removed cancers of the breast and uterus, and tested them on the washed red cells of man, rabbit, sheep, and dog. In every case he obtained hemolysis, and this led him to the belief that the toxin is a general and not a specific hemolysin. He further states that the hemolysin is thermostable, but this conclusion does not seem to be borne out by an examination of his own tables. They show that it takes from three to twelve times as long for complete hemolysis to occur after heating the filtrate to 56° C. for one hour; after heating to 72° the end of the reaction, if it occurred at all, is not even noted. He believes that a better test of the thermostability is the activity of the so-called *Stammacerat*, which had been kept for from seven to eight weeks in thymol-glycerin. Such preservation amounts to antiseptic autolysis, and it is quite clear that such a fluid must contain numerous substances not present in the original tissue or extract. It is, therefore, incorrect to assume that the more active hemolysis in the latter tests is due to the retention of hemolytic substances by the cells. The autolysed fluid probably contained a large number of substances variously affected by heat; nevertheless, the author found that heating to 72° for one hour delayed hemolysis by twenty-four times the period required for the unheated

extract, and heating to 100° C., to two hundred and sixty-four times the period. Kullmann further asserts that these hemolysins are not complex, as proven by their complete absorption by red cells in the cold, with subsequent hemolysis. Unfortunately this conclusion is based on the misconception that the isolation of amboceptor from complement by absorption in the cold is in every case possible — an idea which has been repeatedly corrected by Ehrlich. Finally, he found that the injection of these extracts did not give rise to the production of hemolysins, just as Morgenroth had found of the organ extracts. On the contrary, he found that his injections resulted in the production of a specific hemolysin for human blood, which had all the characters of a typical amboceptor. This result is precisely in accord with known facts regarding the production of specific hemolysins from "blood free" organ extracts. Kullmann's belief that there was too little blood included in this macerate to account for this will hardly be considered tenable in the face of such a result. At all events, the presence of such a powerful hemolysin in the serum would be sufficient to obscure that of an anti-hemolysin. It is, therefore, quite clear that the main conclusions drawn by Kullmann from his own work cannot be accepted in the terms in which he formulates them.

EXPERIMENTAL. *Organ extracts.* — The organs in the present experiments were the kidney and liver of dogs, which were tested as to their hemolytic activity exclusively on the red cells of the same species. The freshly removed organs were at once chopped up and crushed in a mortar. They were mixed with ten times their weight of a physiological salt solution, and either shaken or stirred mechanically for two hours. Their action was tested immediately, or after a further sojourn of ten hours to three days in the ice-box, as stated in case of each experiment. The blood was freshly drawn, generally by aspiration from the external jugular, washed with salt solution by means of the centrifuge, and diluted to a one per cent suspension in physiological salt solution. The serum removed by centrifugation was further used in the experiments.

The first series of experiments demonstrated very clearly the fact that extracts, prepared as above described, exhibit a remarkable diversity of action. Kidney extracts, which had throughout been subjected to the same methods of treatment, manifested either very marked hemolytic activity, or slight activity, or even none at all. There was no apparent cause for these variations, and yet they had been noticeable in the work of previous investigators. Metchnikoff states: "The extract from the spleen is active in only a limited number of cases." Klein found that "the only constant effect was the hemolytic action of the pancreas, and that in a few cases the extract of kidney and of intestinal mucosa also dissolved the red blood cells." Korschun and Morgenroth note that in some cases the dog's spleen showed itself very active, in others absolutely inert. It is evident that a rational explanation of the hemolytic activity of organ extracts must take account of a variability so general and so striking, unless the whole reaction is to be put upon the plane of purely accidental phenomena.

Organ extracts are very evidently not, as they are called, simply the extracts of the organ tissues. In addition, there is mingled with the tissues proper a variable amount of blood, which may, or may not act as a complicating factor. It would, however, seem probable that the presence of a considerable number of red corpuscles in the mixture itself might lead to an absorption of the hemolysins derivable from the tissues, and so vitiate the results of an experiment designed to ascertain their presence. The most thorough-going attempt to exclude this factor was made in the experiments of Korschun and Morgenroth, who exsanguinated their animals; most of the other authors have simply washed the fragments, after cutting the organ into small pieces. Now, it may very easily be demonstrated, either by means of smears or of microscopic sections, that exsanguination is a most imperfect method of getting rid of the blood in an organ. By either method, large numbers of red cells will be found to be present in the organ.

In the present series of experiments, the attempt was made

to render the organ as far as possible bloodless, and then to determine the effect of additions of red cells, serum, and white cells, separately, to the salt solution extracts of such bloodless organs. The method of removing the organs was to free the kidney, which seemed alone practicable for these tests, under ether; dissect out the vessels; cut the arteries distal to a clamp; introduce and tie a canula into a vein, and to pass a prolonged and constant stream of salt solution through the organ. Such a kidney is, to all appearances, bloodless, certainly much more nearly so than is the organ of an exsanguinated animal. Extracts were made of this organ, and were always compared with extracts of the opposite kidney, taken out without the preliminary perfusion.

The action of bloodless and of bloody kidney extracts, as they are denominated in the following experiments, are always markedly different.

The experiments given in the following table are types of a series. To one cubic centimeter of a one per cent suspension of red cells there has been added one cubic centimeter of organ extract in the dilutions given in the left-hand column. The tubes were left in the incubator for two hours, and then in the ice-box for sixteen hours. The table shows the degree of hemolysis at the end of this period.

	Dog 1.		Dog 2.		Dog 3.	
	Bloodless.	Bloody.	Bloodless.	Bloody.	Bloodless.	Bloody.
1- 4...	o	++	+	++	Slight.	o
1- 8...	Slight.	+	o	++	"	o
1-16...	"	+	o	+	"	o
1-32...	"	+	o	+	"	Slight.
1-64...	+	+	o	Slight.	"	"

These experiments indicate that the presence of blood in the organ extract may make a very considerable difference in the end reaction; and they also show that the end reaction is not always affected by this factor in an identical fashion,

although as a rule it increases the hemolytic power of the extract. The last two of the series above quoted may be taken as the type of a series in which it appears that the bloody organ may contain an inhibitory factor, which completely prevents the occurrence of hemolysis in the lower dilutions. The possibility that the increased hemolysis in bloody organs may be due to the presence of a large number of red cells, those present in the organ being superadded to those present in the one per cent emulsion, may be at once disposed of, in view of the fact that the centrifuged extracts, in which no red cells are present, manifest the same differences.

It seemed necessary, therefore, to make a further analysis of the elements concerned. For this purpose, the influence of red cells, white cells, and serum upon the reaction had to be separately investigated. The white cells were obtained by inducing subcutaneous turpentine abscesses in dogs; three or four days later the pus was aspirated, and the action of the pus cells, and also of a sodium chloride extract of the pus cells, was ascertained by adding them to the bloodless organ extract. As an example of the effect of the addition of these various elements to the organ extracts, the following experiment is cited: To separate equal portions of an extract from a bloodless kidney there was added in one case, washed red cells; in a second, serum; in a third, extract of white cells, and in a fourth, full blood. After digesting in the refrigerator for sixteen hours, the clear supernatant fluid was drawn off and its hemolytic activity tested on a one per cent emulsion of red cells. The plain extract was markedly hemolytic. The portion to which the red cells had been previously added was slightly hemolytic, while the portions containing white cell extract, serum, or full blood were inactive. It is therefore apparent that the various constituents of the blood affect the hemolytic activity of kidney extracts in very different fashion.

Serum. — The addition of serum inhibits the hemolytic reaction, and this even when added in very small amounts. The same fact has been noted by Korschun and Morgenroth,

and by Micheli and Donati, the former of whom attribute it to "the existence of a complex of substances having an anti-reactive action, and not to anti-bodies in the proper sense." In this connection it is interesting to note the results of Opie, and of Mueller and Jochman, who found that serum when added to leucocytes inhibits their digestive activity. Morgenroth, however, found that serum retained this power even when heated to the boiling point, whereas, according to Opie, it loses it at 75° C.

Full blood. — The addition of the full blood to the bloodless organ extracts also results in an inhibition of the hemolytic activity of the supernatant fluid.

Red cells. — The addition of washed red cells in quantity to the organ extract diminished its hemolytic activity. This may be explained as due to the absorption of the hemolysins of the extract by the red cells, an interpretation which is amply supported by other experiments subsequently cited.

Absorption by the excess of red cells and the existence of the inhibitory factors in the serum doubtless explains the fact that the extracts of bloody organs frequently fail to hemolyze or do so only in the higher dilutions.

The experiments hitherto cited, however, offer no explanation of the fact that the bloody extracts, as a rule, hemolyze much more strongly than do the bloodless extracts. If the extracts from blood-containing organs are examined after removal of all solid particles, including red cells, by centrifugation, they are found to be strongly stained by hemoglobin, demonstrating the disintegration and solution of enormous numbers of red cells. This is due to the process of grinding and stirring to which such organs must be submitted in the preparation of the extracts. The substances, which have been denominated collectively as R.B.C.D. (red blood cell derivative), should, on the analogy of the previous experiments, be separately prepared, and then added to the extracts of bloodless organs, in order to test their effect upon hemolytic activity. The following method of preparation of R.B.C.D. was adopted: One-half cubic centimeter of washed red cells was dissolved in one hundred cubic centimeters of

distilled water, and to this was added one hundred cubic centimeters of a 1.7 per cent salt solution, which makes a one-fourth per cent solution of red cells in normal salt. A somewhat more effective solution can be obtained by extracting red cells in normal salt for several days in the cold. As the result of a large number of experiments in which the effect of the addition of R.B.C.D. to various organ extracts has been tested the following conclusions have been drawn:

1. R.B.C.D. alone possesses no hemolytic power above that of a normal salt solution.

2. R.B.C.D. added to the extract of a bloodless organ increases the hemolytic activity very materially in nearly every case.

3. An inert kidney extract is not invariably activated by R.B.C.D. This is due to the presence of some complicating factor such as the admixture of blood.

4. Occasionally there is a positive reaction only in the higher dilutions, a phenomenon to be explained by the attenuation, in higher dilutions, of the anti-hemolytic constituents such as the serum. (The activating effect of R.B.C.D. has already been noticed by Polk,¹⁴ who found that it markedly increased the hemolytic power of serum.)

It is therefore quite clear that the final hemolytic effect of the extract from a bloody kidney will depend upon a resultant of forces, of which the organ extract and the R.B.C.D. act in a positive sense, the serum and the W.B.C.D. in a negative manner. The reactions are, on this account, very complex, and the results can never be foretold. Herein lies the explanation of the variability in the action of organ extracts.

If the hemolysis is occasionally increased, and always hastened, by the presence of R.B.C.D. in bloody organ extracts, the question arises whether this element is not an essential factor in every hemolytic reaction by means of such extracts. That this might very well be the case is evident from the fact that that R.B.C.D. must necessarily spontaneously appear in every reaction in which an emulsion of red

cells is employed. It is therefore practically impossible to exclude this element, and extremely difficult to test its part in the reaction. The following experiment, performed upon the model of Ehrlich's well-known reactions, indicates indirectly the manner of interaction:

Experiment 341. — Five cubic centimeters of extract of bloodless kidney is added to the same amount of a fresh one per cent emulsion of red cells. These were left in the incubator for one hour and then centrifuged. The supernatant fluid was added to an equivalent amount of red cells; in this no hemolysis occurred. The sedimented cells were mixed with five cubic centimeters of salt solution, and half of them added to an equal amount of R.B.C.D., the other half left in the salt solution; the former hemolysed strongly, the latter only slightly. A more elaborate experiment on the same type was done with tumors with the same result; it is detailed under another heading (tumor extracts).

These experiments indicate the existence of a double reaction, in which the hemolytic element of the organ extract first unites with the blood cells, but is incapable by itself of producing their solution; the reaction is completed by the addition of R.B.C.D. Further, the organ extract may be exhausted of its hemolysin by absorption and is then inert. There is here an apparent analogy with the hemolyses of the "amboceptor-complement" type. It may here be pointed out that a similar function of the red cells has been discovered by Spangaro.¹⁸ He found that the destruction of anthrax bacilli by the serum is only accomplished in the presence of red cells. Polk's statement has already been cited. The objection raised by Kullmann to this conception of organ hemolysis in general — namely, that it was completed in the cold — has been noticed and disposed of. Much more serious are the objections urged by Korschun and Morgenroth, who also made an experiment to determine the possibility of separating a hypothetical amboceptor and complement. In summarizing their experiment, they make the following statement: "We see, therefore, that at 0° the single solvent dose has been completely anchored by the blood cells and that after centrifuging this leads to complete solution at higher temperatures; double the solvent dose is

still completely anchored by the blood cells. This condition of affairs does not at all correspond to the behavior of the complex hemolysis of serum." More recent publications, however, have withdrawn the framework which supports their conclusion. In an article on "Methods of Studying Hemolysins" Morgenroth¹⁸ states: "Ehrlich and Morgenroth have demonstrated that the binding capacity of red cells varies to an extraordinary degree. While in many combinations the blood cells combine with just that amount of amboceptor, which on the addition of suitable complement leads to their complete solution (amboceptor unit), it was found that in numerous other cases the blood cells are able to take up as high as one hundred single solvent doses of amboceptor."

The thermostability of organ extracts has been defended by Korschun and Morgenroth, and denied by Metchnikoff and by Tarashevitch. These discrepancies have been attributed by some of the authors to differences of technic, but the fact that they have frequently manifested themselves in the same manner in a long series of experiments conducted in identically the same fashion demonstrates that some other factor must be responsible for the variability. As has been shown, there are a number of factors concerned in the reaction, the existence of which had not been previously determined; inasmuch as these factors are partly hemolytic and partly anti-hemolytic, it is obvious that the end result of heating a mixture of them will be in considerable part determined by their individual reaction to that procedure. The various factors were, therefore, tested alone upon red cells, after subjecting them to 50°, 75°, and 100°, for twenty minutes. It was found that R.B.C.D., which is, of course, non-hemolytic, gains no hemolytic power by being subjected to heat. W.B.C.D., in dilutions such as to induce no hemolysis, likewise does not acquire that power by the action of heat. Boiling the organ extract itself markedly diminishes its hemolytic activity, but it is still capable of being activated by the addition of fresh R.B.C.D. It is pointed out that the loss of R.B.C.D. by boiling is always replaceable by

that which soaks out of the red cells employed in the reaction.

STUDY OF TUMOR EXTRACTS.— The material made use of was derived from dogs which had been artificially inoculated with a tumor of the sarcoma group, which has been fully described by Ewing.² A considerable number of these tumors, in various stages of growth, were removed for the purposes of these experiments.

The tumors of strain I. were artificial implantations from a sarcoma of the vagina. Strain II. was a tumor removed from the breast of a bitch and belonged to the carcinoma group. The excised tumors were immediately subjected to the same manipulations as were the organs in the previous series of experiments. The dilutions, 1:10 of salt solution, were also in the same ratio.

A fact which at once became prominent was the marked difference in the hemolytic power of necrotic as compared with non-necrotic tumors of the same strain, or of necrotic and non-necrotic portions of the same tumor. This fact is illustrated by the following experiments: Two tumors were removed from the same animal, one of the tumors being necrotic, the other firm and hard. The extract of the former proved to be very slightly hemolytic, while the latter hemolysed in a dilution of one to forty after two and one-half hours in the incubator, and in a dilution of one to six hundred and forty after twenty hours more in the ice-box. It was this fact which instigated a series of experiments upon autolysed organs and tumors.

It very soon became quite clear that the tumor extracts were as a rule much more active than the kidney extracts. This result is possibly attributable to their higher cellular content, possibly to the fact that the tumor extracts also contained blood. This admixture of blood could not, of course, be avoided as in the case of the kidneys; on the other hand, these tumors are so poorly vascularized that the proportion of blood in the extracts is relatively much smaller than in the organs. The main facts regarding the interaction of

tumor extract and of the various constituents of the blood in the production of hemolysis, as they were found to maintain in the case of organ extracts, were verified also in the case of tumors.

TUMOR EXTRACTS AND R.B.C.D. — As the result of a considerable number of experiments it has been found that R.B.C.D. increases the action of non-necrotic tumor extracts to a very marked degree, but that boiling the R.B.C.D. practically inhibits its power. Tumor extract alone acts much more weakly and slowly. R.B.C.D. also has no hemolytic power when added alone to the red cells.

The manner of interaction of these factors is illustrated in the following set of experiments :

- 351. One per cent blood + salt solution, equal parts.
- 352. One per cent blood + R.B.C.D., equal parts.
- 353. One per cent blood which had been subjected to the action of tumor extract for one hour in the incubator and then centrifuged, + salt solution, equal parts.
- 354. One per cent blood as in 353, + R.B.C.D., equal parts.
- 355. One per cent blood + absorbed tumor extract, equal parts.
- 357. One per cent blood + tumor extract.

The hemolysis in Experiment 357 was marked ; in Experiment 354 it was very strong ; in all the other experiments the reaction was very slight. Thus it is evident that, as in the case of organ extracts, the R.B.C.D. serves to complete the hemolysis of the tumor extract, but is in itself inactive.

The effect of the addition of serum was illustrated in Experiment 356, in which equal parts of serum and of tumor extract were added in one per cent blood ; the hemolysis was very slight.

The addition of a dilute emulsion of leucocytes was tested twice, and in both cases produced a marked diminution of hemolytic activity in the tumor extract.

Thermostability. — In a long series of experiments upon the effect of various degrees of heat on the hemolytic activity of tumor extracts, it was found that the reactions are

extremely complex — much more so than is the case with organ extracts. The cause of this complexity is, in all probability, the admixture of blood. At all events, subjection to a temperature above 70° C. usually results in the formation of an entirely new set of bodies, which act as anti-hemolysins, and may sometimes be removed by dialysis or by filtration. These bodies may be compared to the auxilysins of Manwaring. It seems, on the whole, that extracts of tumors, like those of organs, retain their hemolytic power, in spite of subjection to a boiling temperature, and that the boiled hemolysins are likewise capable of "activation" by the extract of red cells. The individual experiments are too long and too complex for detailed repetition.

Thus it may be said that non-necrotic tumors are similar in their hemolytic action to organs. They possess a hemolysin which is cocto-stable, and which is complemented by the extract of red cells; which is, moreover, inhibited by serum and by an emulsion of leucocytes. The apparent differences in action are probably due to the admixture of blood, which tends to obscure and to complicate these reactions.

Action of necrotic tumors. — When it was found that the necrotic portions of tumors were so much more active than were the undegenerated portions, the attempt was made to compare this condition to that found in the organ proper. This was done both by investigating the action of necrotic organs, and by experiments in autolysis. An experimental necrosis of the kidney was produced. The organ was approached by the transperitoneal route, its pedicle ligated, and then dropped back into place again. After a variable interval of time the animal was killed and the organ extracted in the same manner as previously described. An extract of such a necrotic organ is hemolytic to a degree far exceeding that of the extracts hitherto considered. In the case of one kidney, removed after an interval of five days, the ten per cent extract was hemolytic in a dilution of 1:3200. Moreover, these extracts are entirely unaffected by heat, and

are only slightly inhibited either by serum or by W.B.C.D. The fact that they are different in nature from the extracts of normal organs can very easily be demonstrated by dialysing them for a number of days, which demonstrates that the hemolysins are diffusible. In the extracts hitherto considered, however, this is not the case.

Autolysis was first likened by F. Mueller to necrosis in the body, and this comparison has frequently been verified. If, now, normal kidneys or tumors be autolysed in chloroform water (1 to 10), the chloroform then blown out, and the autolysed fluid tested on red cells, it will be found that it differs in certain particulars from the normal extracts. The autolysed fluid hemolyses, as a rule much more strongly than the simple extract. In addition to the hemolysins it contains a series of anti-hemolysins which may sometimes be removed by passage through a Berkefeld filter. In the latter case, the filtered fluid hemolyses more strongly than the unfiltered. In other respects, the autolysed fluid resembles the necrotic extract, in that its action is not affected by the addition of the constituents of the blood.

CLINICAL DATA. — In considering the applicability of the facts elicited by the foregoing experiments to clinical conditions, it is well, in the first place, to remember that the action of hemolysins is probably only one of the factors in the production of the secondary anemias of malignant tumors. Unfortunately, there are in existence but very few observations which suggest the activity *intra vitam* of the hemolytic factors, the action of which *in vitro* has been demonstrated by the preceding experiments. The occurrence in tissues, such as the kidney, of potential hemolysins, would seem to be a fact without further clinical significance, inasmuch as it is impossible to see how such elements could go into solution in the body fluids. Whether this factor enters into the causation of the anemias of tumors is a question for the solution of which there are too few data. On the other hand, the hemolytic activity of necrotic tissues might very conceivably be

of great clinical importance. Necrotic tissues, even if not malignant, such as uterine fibroids, tend to produce a very appreciable deterioration of the blood. The supervention of degenerative processes in tumors is always the forerunner of a marked increase in the anemia and cachexia. Dr. Beebe informs me that in his series of artificial tumors, cachexia and anemia are very much more marked in those dogs in which the tumors have broken down. Whether the leucocytes and the serum exercise their protective power *in vivo* is also a matter of doubt, although it is a striking fact that areas of infarct and necrosis are always demarcated by a dense wall of leucocytes and the exudation of serum.

L. Bard,¹ in 1901, made a noteworthy contribution to the hemolytic power of cancerous exudates. He found that pleuritic effusions, originating from cancers of the lung, in cases in which the cancerous masses themselves project into the pleura, evince a hemolytic effect upon the red cells contained in the fluid. The latter is discolored by the dissolved hemoglobin, and the red cells are broken up and distorted. In three such cases this was found to hold, in contrast with numerous cases of hemorrhagic pleurisy of tuberculous, septic, or traumatic origin, in which there was no hemolysis of the contained cells. Moreover, non-hemorrhagic pleurisies of cancerous origin do not possess hemolytic power when tested with fresh blood drawn from the finger tip. In a case of ovarian cystoma, with malignant degeneration, it was found that certain of the cysts contained a hemorrhagic and *hemolysed* fluid, others a serous liquid. The former class of cysts, upon closer examination, revealed malignant processes in the wall, while the serous liquid possessed no hemolytic action on fresh blood. Analogous conditions were noted in a case of malignant ascites, and of cancer of the kidney with hematuria. It is evident that the hemolytic fluid in these cases contained both tumor extract and R.B.C.D. Bard's own interpretation must be sought in the original.

Milian,¹¹ in 1901, reported a case of hemolysis in a pleuro-pulmonary sarcoma with hemorrhagic exudation. He found a similar condition in a case of gangrene of the lung, but

confirms the observation of Bard that none of the other forms of pleurisy possess hemolytic power.

Certain observations made upon the blood and the exudations of carcinomatous individuals are of interest in this connection. Lang⁷ investigated the resistance of the red cells of cancerous individuals to hypotonic and hypertonic solutions of sodium chloride, of various concentrations. He found that early or well nourished cases of malignant tumor show no change in resistance. In advanced cases, the red cells evince a much more marked resistance than is normal. They have a resistance-zone of 111-121, as compared to a zone of 42-63 in normal individuals. Janowsky,⁸ who found this resistance most marked in advanced cases of gastric cancer, was the first to attribute it to a specific tumor effect. This argument has been accepted by Schmiedlechner; he puts it in the following terms: "It may be assumed that cancers discharge a toxin into the circulation. This causes tissue destruction; it also has hemolytic power — witness the anemia and urobilinuria. The increased resistance of the red cells represents the organic reaction to this poison (immunity)." Unfortunately, it is not quite clear why immunity to a hemolysin should take the form of an increased resistance to anistonic solutions of sodium chloride. The observation was confirmed in 1906 by del Conte.

SUMMARY.

Extracts of normal organs. — I. Extracts of normal organs (liver, kidney) hemolyse red cells of the same species of animal (isohemolysins), and even of the same animal (autohemolysins), but the hemolytic power varies considerably, and may be altogether absent. Again, there may be hemolysis at a high dilution of an extract, but none at low dilutions — a zone of optimum concentration.

II. If the organ (kidney in experiments) is thoroughly freed from blood, the hemolytic activity of its extracts is to a great extent lost, and may disappear altogether.

III. The iso- or auto-hemolytic power is due in part to the blood contained in the organ.

IV. Addition of entire blood, or, separately, of leucocytes, and serum, to the bloodless organ extracts, however, diminishes whatever hemolytic activity the extracts may possess.

V. But addition of red blood cell extract (R.B.C.D.) will increase (activate) the hemolytic power of bloodless organ extracts.

VI. In bloody extracts it is the R.B.C.D. (analogue of the complement) plus the hemolytic principle of the organ extract (analogue of the amboceptor) which causes hemolysis.

VII. In bloody extracts there is also an anti-hemolytic principle (serum, etc.) which may inhibit hemolysis in low dilutions, while permitting it in the higher.

VIII. This complexity of factors accounts for the great variability in the hemolytic power of bloodless organ extracts. This power probably depends to a great extent on the degree to which the red cells have been broken down during the process of extraction.

IX. Experiments have shown that the hemolytic principle (amboceptor analogue) of organ extracts can be anchored to red cells. The red cells thus sensitized can be washed and then hemolysed by the addition of R.B.C.D. (complement analogue), which latter is of itself inactive.

Tumors. — I. Tumors differ greatly in their hemolytic activity, according to whether they are necrotic or non-necrotic.

II. Non-necrotic tumors are much less autohemolytic than are necrotic tumors.

III. The hemolytic activity of non-necrotic tumors can be increased by addition of R.B.C.D. Extracts of such tumors are in every respect comparable to extracts of normal organs, but are more active.

IV. The hemolytic activity of necrotic tumors is not increased by the addition of R.B.C.D., nor decreased by serum or leucocytes. In this case the hemolytic principle is entirely different from that of organ extracts or growing tumors and is the result of necrosis (autolysis), with the

formation of simple hemolytic compounds, which are dialysable.

V. The anemias of malignant tumors are probably due in part to the hemolytic and toxic action of such products of necrosis.

[In conclusion my very best thanks are due to Dr. Beebe for his constant encouragement and help during the course of the work.]

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A STUDY OF THE TOXINS OF BACILLUS PRODIGIOSUS.*

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Introduction.—In 1892 a study of the therapeutic effect on malignant tumors of inoculations with the combined sterilized toxic products of the streptococcus of erysipelas and the bacillus prodigiosus was undertaken by Dr. W. B. Coley¹ of New York. Previous to that time he, as well as other investigators, had observed the effect of intercurrent attacks of erysipelas and of inoculations with the streptococcus alone on malignant growths, and Roger² of Paris had in experiments on rabbits used the bacillus prodigiosus to enhance the virulence of streptococcus cultures. For the details of these observations the reader is referred to the original papers.

Through Coley's work extending now over fifteen years, during which time some striking therapeutic results have been obtained by the use of the so-called "mixed toxins," the bacillus prodigiosus has assumed a position of much greater practical importance than a mere laboratory reputation had heretofore accorded to it. Up to the present time, however, no work has been done to determine whether this organism may not in itself play some active rôle in the therapeutic effect produced by the mixed toxins. Dr. Coley states: "The fact that all of my own successes as well as those of other surgeons have been obtained by the combined toxins, not a single permanently successful case having been observed from the use of the erysipelas toxin alone, goes far toward establishing the importance of the bacillus prodigiosus."

The clinical results, therefore, abundantly justify a more extended study of this organism in this connection, and the following investigation has accordingly been undertaken, leading up to a further study, already under way in this

* Received for publication April 12, 1907.

laboratory, of the action of the bacillus prodigiosus alone, and in combination with the streptococcus, in the treatment of rapidly-growing lymphosarcomas in dogs.

Review of literature. — On looking over the literature one finds that little systematic study of the bacillus prodigiosus was undertaken until a comparatively recent date.

A. B. Griffiths³ in 1892, F. Scheurlen⁴ in 1896, and Mary Hefferan⁵ in 1903 investigated carefully the pigment-producing power of the organism, the nature of the pigment, and the conditions favoring its production. These writers agree, and the experience acquired during the present study is in accord with their conclusions, that the essential conditions of pigment production include: a free supply of oxygen; a temperature of about 20° C.; and a medium slightly alkaline to litmus. Mary Hefferan further concludes that, "There is probably little or no correlation between luxuriance or vigor of growth and the power of pigment formation. Hence pigment production does not appear to be essential to the life processes of an organism."

In 1897 Grawitz and de Bary⁶ reported the production of local abscesses in dogs, cats, and with difficulty in rabbits, by subcutaneous inoculations of cultures, whether living or dead, of prodigiosus, the effect being similar to that produced by the inoculation of turpentine. These observations were repeated by Steinhaus⁷ in 1889.

Bertarelli,¹⁵ in 1903, published a somewhat more elaborate study of the toxic action of the organism. Live broth cultures of prodigiosus were injected intraperitoneally into guinea-pigs and found to be toxic in doses of from .8–2.0 cubic centimeters, according to the age of the culture, a fatal result supervening in from eighteen to thirty-six hours. Intravenously the fatal dose was from 1.–1.5 cubic centimeters, while subcutaneously 2.0–2.5 cubic centimeters only occasionally caused death. The subcutaneous inoculation of non-fatal doses caused local scant pus formation, rarely a true abscess. Rabbits were less sensitive to the treatment than were guinea-pigs.

Experiments with culture filtrates, and with suspensions of bacilli killed by heat and subsequently washed, lead him to conclude that the chief toxic effect was due to substances bound up in the bacterial cells. Bertarelli also described a strong hemolytic property in vitro, in the filtrates from his broth cultures.

A. J. Detweiler,⁸ in the laboratory of V. C. Vaughan, prepared a dry powdered prodigiosus germ substance with which he was able to obtain more definite and accurate data as to the toxic power of the bacterial cells. He found that the smallest fatal intraperitoneal dose of such germ powder was one part of powder to ninety thousand parts of body weight of the guinea-pig used, death resulting within twelve hours after inoculation. Post-mortem examination showed some clear fluid, and at times masses of germ substance in the peritoneal cavity; mesenteric vessels injected, gall-bladder greatly distended, "adrenals of a deep red color," lungs congested, but other organs not greatly altered. Where a quantity of the germ substance had escaped under the skin was found no pus, but a clear gelatinous exudate. Detweiler found it possible to establish a certain degree of immunity to the toxin, but beyond a definite point the animals rapidly emaciated and died. Serum from such a partially immune animal in dilution of 1-10 agglutinated prodigiosus bacilli, while an equal dilution of normal guinea-pig serum failed to so act. No attempt was made to break up the bacterial cell nor to extract a specific toxic substance therefrom.

The present investigation may best be described under the following heads:

1. Experiments with broth cultures of *B. prodigiosus*.
2. Experiments with the pure, whole germ substance, both in dry powdered form and in suspensions of bacilli killed by heat, in which the weight of bacterial nitrogen was determined by the Kjeldahl method.
3. Experiments with filtrates from bacillary suspensions previously subjected to procedures designed to break up the bacterial cells. These procedures include: (a) grinding

with liquid air; (*b*) hydrolysis in the autoclave at pressure of twenty pounds; (*c*) hydrolysis in a weak acid (.2 per cent HCl) medium at 70° C.; (*d*) hydrolysis in a weak alkaline (1 per cent NaOH) medium at 70° C.; (*e*) digestion with pepsin in .2 per cent HCl solution; (*f*) digestion with trypsin in .5 per cent Na₂CO₃ solution; (*g*) autolysis at 38° C.

1. Experiments with broth cultures of *B. prodigiosus*.

Since the prodigious fractions of the mixed toxins have, up to the last few months, consisted of broth cultures of the organism, such cultures were used in a series of tentative experiments on rabbits, and subcutaneous inoculations only were tried in this first series.

Broths of various formulæ were employed, the variations consisting in alterations of the usual beef and peptone broth by the addition of rabbit or of sheep serum; by the omission of peptone; by the use of a special so-called "enzyme peptone;" and by the addition of sodium phosphate. No variations in the growth and behavior of the cultures could be correlated at all with these differences in media. A condensed statement of the results of this series of inoculations is given in Table I.

The cultures used were all twenty-one days old, and had been maintained under conditions as nearly as possible identical.

TABLE I.
Results of subcutaneous inoculations of rabbits with broth cultures of prodigiosus and with filtrates from such cultures.

Material used for inoculation.	Previous treatment of material.	Dose.	Temperature reaction.	Local reaction.
Whole culture.	Unheated, living.	.2-.3 cc.	Rise of 1°.	Brawny swelling. No necrosis.
Whole culture.	Heated to 70° C. for ½ hour.	.2-.5 cc.	Rise of 2°-4°.	Brawny indurated swelling followed by central necrosis.
Whole culture.	Heated to 55° C. for ½ hour.	.2-.5 cc.	No rise—rise of 2°.	No reaction after smaller dose. Brawny swelling after larger dose. No necrosis.
Suspension of washed bacilli.	Unheated.	.1 cc.	Rise of 1°.	Brawny swelling. No necrosis.
Berkfeld filtrate of broth culture.	Unheated culture.	.5 cc.	Rise of 3°.	Slight puffiness only, 3 cases. Inflammation and necrosis, 3 cases.
Same.	Culture heated to 70° for ½ hour before filtering.	.5 cc.	Rise of 2°.	Slight swelling and inflammation. Necrosis in 1 case only.
Same.	Culture heated to 55° for ½ hour before filtering.	.4-.5 cc.	Rise of 2°-4°.	No reaction in 4 cases. Slight swelling and inflammation in 2 cases. Necrosis in 1 case.
Control: beef peptone broth, } sterile.4 cc.	No rise.	No reaction, 2 cases.

It will be noted that the filtrates did in a few cases produce some local reaction, but by no means with the regularity and to the same degree of inflammation as did the whole cultures. The small quantity of filterable toxin obtained from a broth culture is doubtless to be ascribed to the breaking down of the dead cells present.

The filtrate from a broth culture proved hemolytic for dog red cells in a dilution of one to four only. It seems improbable that a reaction so slight was called a strong hemolytic action by Bertarelli, who, however, fails to give the dilution of his filtrate.

2. Experiments with the whole germ substance in dry powdered form, and in suspensions of bacilli killed by heat in which the weight of bacterial nitrogen was determined by the Kjeldahl method.

The difficulty of obtaining any considerable quantity of bacterial substance, and of measuring apart from the medium such as was present, lead to the abandonment of cultivation in broth. Hereafter, agar cultures were used, and thus there could be obtained masses of germ substance practically pure from which definite quantities of bacillary proteid could be prepared. Unfortunately no bacterial tank of Vaughan's pattern was available, but the large Fernbach culture flasks were found to serve the purpose fairly well. The ordinary two per cent agar medium of a depth of about one centimeter over the bottom of the flask was used. A thinner layer proved insufficient to support a vigorous ten days' growth. The flasks were sown by pouring over the surface of the agar a two days' old broth culture of *B. prodigiosus*, and draining off the surplus fluid. In ten days the thick, bright red pellicle was removed with a glass rod bent to a convenient angle, using a little distilled water when necessary. Employing a mortar and pestle this mass of bacteria was rubbed with more water to a smooth, rather thick suspension. It was then poured slowly, with active stirring, into a large volume of ninety-five per cent alcohol, being thrown down therein as a pinkish white flocculent precipitate. Nearly all of the

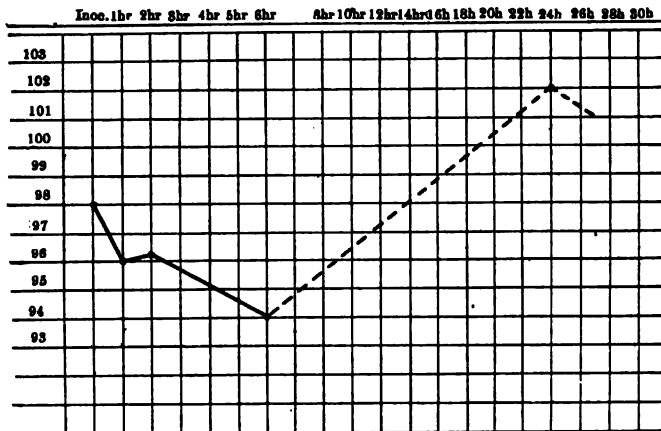
pigment became immediately dissolved in the alcohol. After filtration, and washing with absolute alcohol and ether, the precipitate was dried in the air and ground in a mortar to a fine powder. Such prodigiosus powder was of a pale purplish color, and when smeared with a little water on a slide and stained showed the bulk of the bacterial cells staining well and apparently morphologically unchanged by the treatment. This manner of preparation of the germ powder does not differ materially from that employed by Detweiler, and the toxicity of this product proved very similar to that of his, one milligram of the powder to one hundred thousand milligrams of body weight bringing about a fatal result in nearly every case.

The germ substance in suspensions was prepared also from the agar cultures, the emulsions were made somewhat thinner than those used for precipitation in alcohol, were then bottled, and sterilized at 75° for one hour. As previously noted the method of cultivation and removal from the agar gave the germ substance practically free from any contamination from the medium, and all the nitrogen present in the suspension was, therefore, contained in the bacterial cell. The total nitrogen content of a definite volume of this suspension was determined by the Kjeldahl process, and making use of the fact that one gram of nitrogen represents 6.25 grams of proteid, the proteid content of the bacterial suspensions was readily estimated.

The results of a series of inoculations proved this method of measurement satisfactory for the work with the whole germ, and quite comparable to the actual weighing of the dry germ substance. The toxicity of the two preparations was practically identical, an intraperitoneal dose of one part of bacterial nitrogen to six hundred thousand parts of body weight being, with few exceptions, fatal. In the work with the whole germ substance, therefore, in either of the above-described preparations, the doses were reckoned in parts of bacterial nitrogen to parts of body weight.

The symptoms exhibited by a guinea-pig after receiving a fatal dose of prodigiosus germ substance begin to manifest

FIGURE II. — Temperature Curve of Guinea-pig after Intraperitoneal Inoculation of a Non-fatal Dose of Prodigiosus Powder.



An observation made at this time, and returned to with interest later, was the fact that in a number of instances pigs which had received a severe but not fatal dose of the germ substance recovered from the immediate effect of the toxin, but died in an emaciated condition a week or ten days later.

The post-mortem findings in a pig killed by prodigiosus suspensions are as follows: a small quantity of clear straw-colored or blood tinged fluid is usually present in the peritoneal cavity, but the peritoneum preserves its normal glossy appearance; the liver may be slightly pale, or in other cases congested, and wide distention of the gall-bladder with clear fluid bile is quite constant; the spleen is often mottled, deeply congested areas contrasting with others of marked pallor, or in those cases in which death occurs a week or more after inoculation the organ is exceedingly pale throughout; the kidneys appear unaltered or only slightly congested; the adrenals are uniformly deeply congested and often hemorrhagic. This hyperemia of the adrenals was noted also by Detweiler in his work with prodigiosus, and has been observed by Gelston and others in pigs killed by

diphtheria germ substance, and by Torrey in this laboratory in pigs killed by gonococcus cultures.

Having now obtained and proved a preparation of the prodigious germ substance, which could be handled with some degree of accuracy as to dosage, a second series of subcutaneous inoculations was undertaken. Non-fatal doses of powder suspensions were used, the local lesions produced were removed at intervals, after killing the animals with ether, and the gross and histologic changes studied.

After such inoculation the tissues surrounding the needle puncture became edematous and swollen and, after several hours, induration, and at times active inflammation, was noted. The inflammation rarely persisted for any length of time, but the induration continued for several days before absorption began. Occasionally, slight superficial necrosis was observed at the center of the inflammatory area. Gross sections through such an early edematous lesion showed infiltration with a clear yellowish or hemorrhagic gelatinous exudate. Section through a week-old lesion showed a circumscribed area resembling coagulation necrosis.

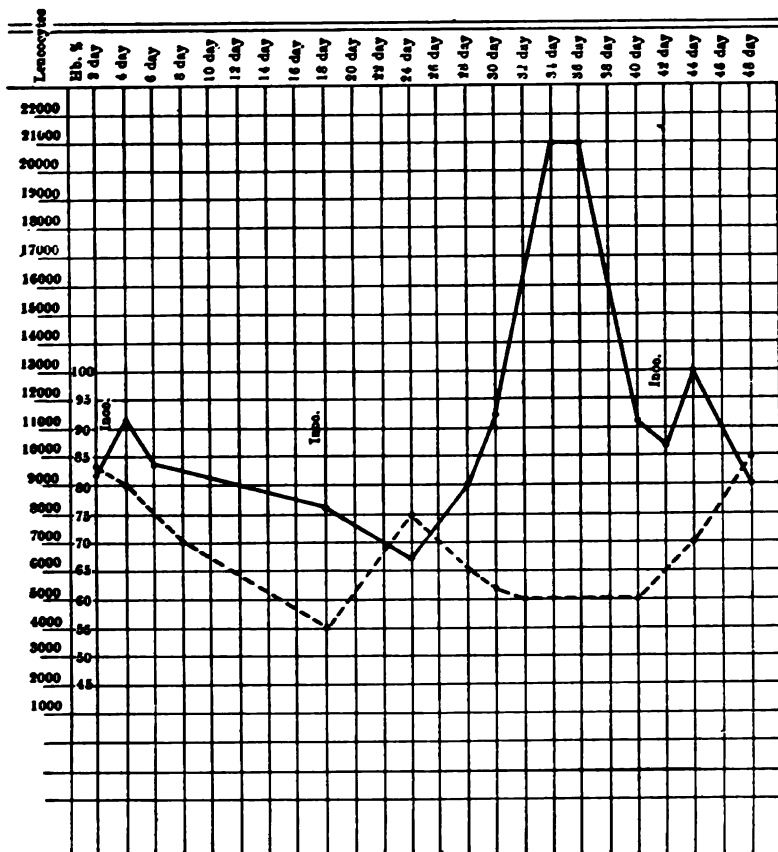
Microscopically nothing definite was noted in the earliest lesions (one hour after inoculation), but gradually a migration of polynuclear leucocytes to the site of inoculation occurred, and a thick layer of these surrounded the caseous center. Detweiler observed the gelatinous subcutaneous exudate, and no doubt the later caseous accumulations correspond to the abscesses of Grawitz and de Bary and Steinhäus, and the "scant pus formation" of Bertarelli. The facts agree with Coley's suggestion as to the nature of the local action of the mixed toxins, "a coagulation necrosis with a local leucocytosis."

Endeavors to establish immunity to the prodigious toxins in guinea-pigs and rabbits have met with little success. Nearly all of the animals used appeared rather to acquire a hypersensitiveness, and died after the second or third inoculation of a dose no greater than that used at the start, and only one pig was finally brought to a point of surviving the usual fatal dose. During the two months' treatment the

animal lost weight steadily, and in six days following the last inoculation fell from three hundred and seven to one hundred and forty-three grams. The pig was then killed to obtain blood serum, which was found to agglutinate suspensions of prodigiosus bacilli in dilution of one to twenty. The serum of a dog, which was receiving repeated subcutaneous inoculations of similar suspensions, gave a positive agglutination reaction at a dilution of one to sixty-four, though a severe temperature reaction occurring after each inoculation (the dose was not increased), did not indicate any high degree of immunity or even of tolerance. Although these agglutination tests showed a positive reaction at a greater dilution than was obtained by Detweiler, the general negative result of this phase of the work is in accord with his observations.

A series of blood examinations, enumeration of red and white cells, hemoglobin estimation (Miescher-Fleischl), and differential leucocyte count was made on animals receiving repeated small doses of prodigiosus suspensions. The results showed an increasing leucocytosis, with a slow falling off in the number of red cells and in the hemoglobin percentage. Figure III. expresses in the form of a curve these changes in a rabbit in whose case the dosage was kept very low so as to avoid all risk of a fatal issue.

FIGURE III. — Leucocyte and Hemoglobin Curves in a Rabbit receiving Repeated Small Doses of *Prodigiosus*.



Dogs undergoing treatment of tumors show similar blood changes. When inoculations were omitted for a week or more the blood gradually returned to the normal condition.

Experiments up to this point have shown that the bacillus *prodigiosus* contains within the germ cell some poison or poisons capable of causing death in animals in very small doses. The convulsive symptoms and the post-mortem condition of the organs point to an overwhelming toxemia of the nervous mechanisms in those animals dying within

twenty-four hours of inoculation, while in those dying a week or more later the symptoms of emaciation and general weakness, with the post-mortem pallor of the organs, and distention of the gall-bladder with fluid bile, indicate, as a secondary effect, a progressive blood destruction. With this later effect may be correlated also the blood changes noted in a preceding paragraph.

The question arises — are these two effects, the overwhelming toxemia of the nervous system and the slowly developing anemia in an animal withstanding an immediate fatal issue, to be ascribed to a single substance, in the second case acting over a longer period of time, or are we dealing with a mixture of toxic bodies? To throw further light on the matter the breaking up of the bacterial cell was undertaken.

3. Experiments with filtrates from bacterial suspensions previously subjected to procedures designed to break up the germ cells.

The study of endotoxins, and their preparation by various methods from the germ cells, has been undertaken extensively by V. C. Vaughan,⁹ V. C. Vaughan, Jr.,¹⁰ Wheeler,¹¹ Gelston,¹² J. W. Vaughan,¹³ and others. In 1901 Vaughan stated that the toxin of the colon bacillus, "as separated from the cell wall by digestion with hydrochloric acid and pepsin is markedly active." In the later work from his laboratory, however, the methods used for breaking up the germ cells of anthrax, colon, and diphtheria bacilli have been far more drastic. The bacterial powder was subjected to the action of one per cent sulphuric acid at a temperature of 110° C. for ten minutes; or was boiled for "one hour on the water bath under a reflux condenser with from fifteen to twenty times its weight of absolute alcohol in which two per cent by weight of pure sodium hydrate had been previously dissolved." From the filtrates obtained after these procedures, the toxic substances were precipitated out by alcohol. V. C. Vaughan, Jr., concludes his study of such a poisonous body derived from the colon bacillus with the statement that "This

intracellular poison is the poison which causes death in animals inoculated with cultures of the living colon bacillus."

That poisonous bodies can be obtained from bacterial cells by such drastic chemical methods is unquestionable, but whether the resulting bodies are identical with those produced physiologically in the living body, seems open to legitimate doubt.

In 1904 McFadyen worked with the typhoid bacillus, and later with the pneumococcus, grinding the bacterial suspensions with liquid air and subsequently extracting with weak alkali solutions. Thus he succeeded in obtaining from his filtrates markedly toxic soluble bodies. This method is less objectionable than the chemical method of Vaughan, for it is quite conceivable that the mechanical rupturing of the cell body does not in any way alter its chemical nature, yet does render the proteid substances more accessible to the action of the alkaline solution used for extraction. For the purposes of the present study it was thought desirable to use procedures more nearly comparable to those taking place in vivo. At the start certain methods of hydrolysis, not within the limits of the physiological, were tried, but were subsequently abandoned, as those most resembling the body processes were found to yield soluble products of more definite toxic action.

a. An attempt was made to repeat the cold grinding method of McFadyen, and, for his interest and time spent in using the liquid air apparatus at the College of Physicians and Surgeons, we are very greatly indebted to Dr. A. N. Richards. Owing to certain mechanical difficulties which arose, the attempt was unsuccessful and was not repeated.

b. Suspensions in .2 per cent sodium bicarbonate of the dry powdered germ substance were heated in the autoclave under a pressure of twenty pounds for two hours. Repeated inoculations of guinea-pigs with samples of these suspensions previous to the hydrolysis gave fatal results in the usual lethal dose. After the hydrolysis inoculations of equivalent and even of larger doses of the whole unfiltered suspensions or of filtrates produced no toxic effect whatever.

c. Suspensions of prodigiosus powder in .4 per cent hydrochloric acid were subjected to a temperature of 70° C. for three hours. Inoculations in the usual dose of the unfiltered hydrolyzed suspension and of the filtrate caused a slight lowering of body temperature, followed, however, by a prompt return to normal.

d. Suspensions of prodigiosus powder in one per cent sodium hydroxide were subjected to a temperature of 70° C. for three hours. After hydrolysis the suspension was neutralized with hydrochloric acid. Inoculation with the unfiltered product in the usual dose caused death in one animal, but was followed by recovery in others. Inoculation with the filtrate caused a lowering of the body temperature followed by immediate recovery, though death of one animal followed five days later.

e. To suspensions of prodigiosus powder in .2 per cent hydrochloric acid a sufficient quantity of pepsin was added and digestion allowed to proceed for five hours at 37° C. The digested product was neutralized and heated quickly to boiling for ten minutes to destroy the enzyme. Inoculations with the unfiltered product caused death within twenty hours in one animal, and in two others death followed some days later. Inoculations with the filtrate produced slight lowering of body temperature followed by recovery when the equivalent of the usual fatal dose was given. A prompt fatal result, however, followed a dose twice as large.

f. To suspensions of prodigiosus powder in a .2 per cent sodium bicarbonate solution sufficient trypsin was added, and digestion allowed to continue for one and a half hours. The product was neutralized and heated to destroy the enzyme. The filtrate from this digestion, and a substance obtained from it by precipitation with alcohol, caused death in doses about three times as large (three times the nitrogen content) as the usual fatal dose of the whole germ substance.

With the hope of obtaining a greater quantity of this poisonous product other tryptic digestion experiments were undertaken. Suspensions of fresh bacilli were subjected to

the action of the enzyme for longer periods, one, two, and three days, in order to break up a larger quantity of the bacterial bodies. At the end of each period the appropriate suspension was neutralized with hydrochloric acid and filtered through a Berkefeld candle. The filtrates were evaporated to dryness on a water-bath and the residues extracted with absolute alcohol for three hours. There remained undissolved in each case a golden brown substance which was subsequently dissolved in distilled water and designated as the alcohol insoluble fraction, or fraction A.

The clear brown alcoholic extracts were in turn evaporated to dryness on the water-bath, the residues taken up in distilled water and designated as the alcohol soluble fractions — fractions B.

At the same time a similar suspension with sodium bicarbonate and trypsin was arranged for digestion in a parchment bag, which was suspended in a covered jar also containing .2 per cent sodium bicarbonate solution. This digestion was allowed to continue for five days, during which time the fluid outside the bag was removed daily and replaced by fresh solution. This method intensified the digestion by removing the dialyzable products as they formed. The combined dialysates were neutralized and evaporated to dryness, and separated as described above by means of alcohol into fractions A and B.

The concentration of these several preparations in terms of nitrogen was then determined as a guide to dosage and the toxicity tested on guinea-pigs.*

The result of the inoculations showed no gain, but rather a loss in the power of the product to cause a rapidly fatal issue, this loss being most decided in the bag digestion experiment, where the cleavage had proceeded furthest, owing to the removal of the resulting substances. We must not fail to consider here, however, the possibility that the more powerful toxic substance was not dialyzable, and therefore

* In these A fractions it was necessary also to determine the quantity of sodium chloride present as a result of the neutralization, and to dilute the solution to physiological strength before inoculations. Volhardt's method was used.

remained within the bag subject to the enzyme action until destroyed. Baldwin and Levene¹⁴ have shown that proteolytic enzymes, pepsin, papain, and notably trypsin, digest the bacterial toxins of diphtheria and tetanus bacilli, destroying their power.

The A fractions in doses six times as large as the lethal dose of the whole germ substance caused only a slight lowering of body temperature followed by prompt temporary recovery. But of great interest was the observation that nearly every animal died more or less emaciated in from six to twenty-four days after the inoculation. The post-mortem findings showed the usual features, enormously distended gall-bladder, pale spleen, and adrenals considerably congested in the earliest fatal cases, but very much less so after longer survival.

The B fractions in equally large doses caused not even a temporary fall in body temperature, but death followed after several days in most cases, and the post-mortem findings were identical, save that the hyperemia of the adrenals was much less noticeable.

g. Autolysis at 38°.

Suspensions of bacilli in distilled water were placed in the incubator and autolysis allowed to proceed for two weeks. A culture taken from the bottle at the end of this time gave a pure growth of *B. prodigiosus*. It is probable that the density of the suspension gives no favorable opportunity for development of any accidental contamination which might occur. The suspension was then heated to 75° C. for one hour and filtered, and the filtrate divided as described above into an alcohol insoluble fraction A and an alcohol soluble fraction B. The nitrogen content of the solutions then was determined and the toxicity tested on guinea-pigs.

The results were striking. Fraction A caused death almost without exception within twenty-four hours with the usual acute symptoms, rapidly falling temperature and convulsions. This result was obtained with a dose of one part of toxic nitrogen to five hundred and forty thousand parts of body weight. The autopsy showed as the salient points: a little clear straw-colored or red-tinged fluid in the peritoneal cavity, the spleen

or mottled, the adrenals deeply hyperemic and hemorrhagic, and the gall-bladder not at all, or very little, enlarged.

Fraction B produced no immediate ill effect, but rapid loss of weight followed and death occurred in ten days. Post-mortem there was found no fluid in the peritoneal cavity, the spleen was very pale, adrenals little or not at all hyperemic, and the gall-bladder widely distended with yellow or red tinged fluid bile.

The striking hemolytic action of fraction B as compared with that of fraction A was further demonstrated by a series of experiments in vitro. The A tubes showed no hemolysis of dog or of rabbit red cells after eighteen hours, while the B tubes showed hemolysis complete in dilution of 1-100 in two hours. The presence of normal dog serum in the tubes delayed the hemolytic action, and the serum from a dog receiving repeated small doses of B. prodigiosus arrested it entirely.

In comparison with these experiments a series with fractions A and B of the long tryptic digestion filtrates gave interesting results, especially with those from the parchment bag experiment in which the cleavage of proteid was most complete. Both fractions A and B caused complete hemolysis within an hour, and the dog serums mentioned above failed to arrest the action in the slightest degree. It will be remembered that both fractions A and B of this parchment bag filtrate caused death in pigs *apparently* only as a result of the slow blood destruction. The conclusion that fraction B causes death by a slow blood destruction is a tentative one, and is based on the evidence offered by the marked hemolysis in vitro, and by the post-mortem findings.

Fractions A and B of the autolysis filtrate responded to the proteid reactions as follows:

	A.	B.
Precipitation with heat and acetic acid	—	—
Precipitation with nitric acid	—	—
Precipitation with potassium ferrocyanide		
and acetic acid	—	—

	A.	B.
Precipitation with absolute alcohol . . .	+	—
Precipitation with basic lead acetate . . .	+	+
Color reaction — Xanthoproteic . . .	+	+
Millon's reaction . . .	+ slight.	+ intense in the cold.
Biuret — doubtful in both cases on account of the deep color of the solutions.		

There are many points not cleared up by the experiments thus far undertaken. The measurement of the poisonous bodies in the filtrates by estimation of nitrogen is less satisfactory than was the case when the whole germ substance was used. No relation of the fatal dose of the split toxin to that of the whole germ substance from which it originated can be determined thus, for it is probable that a considerable part of the nitrogen present in the filtrate is in a non-poisonous form. Furthermore, there remains always a considerable mass of germ substance not yet broken up by the digestive enzymes or by autolysis. This seems inevitable inasmuch as longer continuance of the process carries the cleavage too far and reduces the substance present to a far less toxic condition.

The true chemical nature of the bodies with which we are dealing can only be determined by much further chemical study. Their exact position in the line of cleavage products, and the relation of the two poisonous bodies to each other, whether the hemolytic substance characteristic of the fraction B is derived from the toxic body of fraction A by further cleavage, as the long digestion experiment might suggest, are questions yet to be elucidated.

To summarize. — 1. It has been shown that the bacterial cell of the bacillus prodigiosus contains highly toxic bodies capable in very small doses of causing death in animals.

2. Inoculations with suspensions of the whole germ substance may cause death within twenty-four hours, the striking symptoms being the rapid fall in body temperature and the occurrence of convulsions, or death may follow from one to three weeks later, preceded by gradually developing anemia and emaciation.

3. Post-mortem changes conspicuously present are

extreme hyperemia of the adrenals in the cases of immediate death, and great pallor of the spleen and wide distention of the gall-bladder with fluid bile, in cases of late death.

4. Subcutaneous inoculation of a non-fatal dose of prodigious suspension produces a local lesion consisting of a central area of coagulation necrosis surrounded by a zone of active round cell infiltration.

5. Of the procedures used to split up the germ cell, omitting the cold grinding experiment (*a*) as inconclusive, it has been shown that: (*b*) hydrolysis in the autoclave totally destroyed all toxic qualities of the prodigious cell; that (*c* and *d*) hydrolysis at 70° C. with weak acid or weak alkali split off very small quantities of toxic substances from the germ cell; that (*e* and *f*) digestion with the enzymes pepsin and trypsin split off somewhat large quantities of filterable poisonous bodies, which, however, had to be administered in large doses, as measured by nitrogen, in order to produce an immediate fatal result. Further, if the tryptic digestion were prolonged, the substance capable of causing the death of a guinea-pig within twenty-four hours disappeared more or less completely, and the filtrates remained capable of but one poisonous action, that of slow blood destruction; finally, that (*g*) autolysis at a temperature of 38° C. for two weeks proved to be the method most effective in setting free from the germ cell soluble toxic substances which pass freely through a Berkefeld filter, and which are capable of producing in guinea-pigs, when injected intraperitoneally, that train of symptoms, fatal result, and post-mortem appearance which have been observed to follow inoculations with the whole germ substance.

6. A solution of these substances it has been shown can be divided chemically into two distinct fractions, and with each fraction can be correlated certain poisonous qualities, the alcohol insoluble fraction being highly toxic while the alcohol soluble fraction is chiefly hemolytic in its action.

The relation of the facts brought out by this study to the preparation of the mixed toxins of streptococcus and prodigious, and to the therapeutic value of the preparation as

tested in the treatment of sarcomas in dogs, will be considered in a later paper.

[It is a pleasure to express my thanks to Dr. S. P. Beebe for his constant interest and many suggestions offered during the progress of this work.]

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AGGLUTININS AND PRECIPITINS IN ANTI-GONOCOCCIC SERUM.*

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In December, 1906, I¹ described the action and method of production of an anti-gonococcic serum, which gave evidence of being of therapeutic value in the treatment of gonorrheal arthritis. At the time, announcement was made of the fact that the serum contained specific agglutinins and precipitins for gonococcus. Since then a detailed investigation into the nature of these anti-bodies has been carried on and the result of this study is embodied in the present paper.

Until very recently the problems concerned in experimental immunity to gonococcus in animals have received scant attention, except as regards the nature of the toxin of this organism. Among the few earlier observations we find that Moltschanoff (1899)² was not able to demonstrate the production of agglutinins in the blood of rabbits inoculated with gonotoxin, and Jundell (1900)³ failed to obtain a clumping of gonococci with the serum of patients with gonococcus metastases.

Several months after the above-mentioned preliminary announcement, Bruckner and Cristéanu⁴ described a serum produced by immunizing a horse to several strains of gonococcus. This agglutinated an emulsion of gonococcus passed through quadruple Chardin filter paper at 1-750 macroscopically, and at 1-2,000 microscopically in twelve hours at 37° C. A little later (December, 1906) Vannod⁵ published similar results. This investigator raised an anti-serum in rabbits by injecting increasing amounts of gonococcus nucleo-proteid. After five inoculations an animal had a titer of 1-200, and after ten, 1-1,000 in three hours. These are the only experiments on this subject which have been found in the literature.

* Received for publication April 12, 1907.

Aside from its general scientific interest, two special questions have been held in view in this work. First, are all strains of gonococci identical, or do they form a group of varieties, as is the case with *B. dysenteriae* and *Streptococcus*? If the latter proved to be the case it would be necessary in producing a therapeutic serum to inject a number of different strains into the animal employed in order to make the serum as far as possible polyvalent. The second immediate question has been the determination of the relation, if any, of the agglutinating power of a serum and its therapeutic potency. It may be said that the experiments point clearly to the conclusion that the gonococcus family is heterogeneous rather than homogeneous. It may, in fact, be divided into several groups, each raising agglutinins which are specific for its own group. The absorption experiments, on which this conclusion is based, are given in detail in the latter portion of this paper. The relation of the therapeutic properties of a serum and its agglutination titer will be discussed in another communication; suffice it to say that the agglutination test has been found to be a reliable index of the therapeutic potency of an anti-gonococcic serum.

Cultures. — The cultures employed in this work have been isolated from various sources. For the sake of perspicuity they are designated by the first ten letters of the alphabet. In the following list the source from which they were isolated and their original names are given :

Culture.	Original Designation.	Origin.
A	"Edna," by E. and H. *.....	Vagina.
B	"Muller," by E. and H.	Urethra.
C	"Keating," by E. and H.....	Urethra.
D	"Gelber," by Wilson	Vagina.
E	"2368 S.," by Wilson	Periurethral abscess.
F	"Vargo," by Wilson	Vagina.
G	"Weston," by E. and H.	Joint.
H	G-14, by E. and H.	Joint.
I	G-12, by E. and H.	Urethra.
J	G-10, by E. and H.	Urethra.

* Kiser and Huntoon.

All of these strains have been identified positively as gonococci. Their growth is typical in ascitic agar and in ascitic broth. They are negative to Gram's stain. On ordinary nutrient beef agar, reaction ± 7 to phenolphthalein, cultures A, B, D, E, I, and J grow very feebly or not at all; cultures C, F, G, and H thrive better on this medium, but in no case is the growth as luxuriant as on ascitic agar. Several cultures, which refused to grow without ascitic fluid when first isolated, after several months' cultivation showed a slight growth on ordinary media. The addition of glucose or glycerine to media does not, according to my experience, cause an increase of growth, but if anything has an inhibiting influence.

Media. — Until recently there has been a general impression that gonococcus will not grow on media unless some human fluid is added to it. Neisser (1889),⁶ Kiefer (1896),⁷ Wassermann (1898),⁸ and Fränkel (1899)⁹ have laid emphasis on this point as a distinguishing one between gonococcus and other Gram-negative diplococci, especially when first isolated from pus. Wertheim (1892)¹⁰ seems to have been the first to recognize the fact that this distinction

is not altogether valid. He found that gonococcus would thrive on both plain and glycerine agar, although very much less luxuriantly than on coagulated human serum. Ten years later Wildbolz (1902),¹¹ published the results of some experiments on the cultivation of various strains of gonococcus on ordinary media. Out of twenty cultures he found that sixteen grew moderately well on plain agar, slightly alkaline to litmus, and four did not. These strains had been growing for several generations on serum agar before being transplanted to the plain medium. To Thalmann (1900, 1902),^{12, 13} however, belongs the credit of devising a medium on which all strains of gonococci may be induced to grow well without the addition of any human fluid. The secret, he thinks, lies in the proper reaction. Although gonococcus will grow on litmus acid and litmus neutral media, it reaches its optimum between this and neutralization to phenolphthalein, viz., slight alkalinity to litmus. As his medium is very useful in the study of gonococcus and has been used extensively in the experiments recorded here, a description of the method he uses in its preparation may be given:

Place five hundred grams of chopped beef in one liter of distilled water. Boil over free flame fifteen minutes. Pass through flannel. Add peptone one per cent and NaCl .5 per cent. Bring to a boil over flame. Cool and filter. Heat the filtrate and add 1.5 per cent agar. Boil for forty-five minutes in a bath of saturated NaCl solution. Neutralize two-thirds of the medium to phenolphthalein and add it to the remaining unneutralized portion. This brings the reaction of the medium to the proper point, about +.1 to phenolphthalein. It may generally be filtered clear without the use of albumen. If cloudy, the white of one egg to the liter is sufficient to clear it. The proper reaction cannot be obtained with certainty by using litmus as an indicator.

This medium has been used with success by Ströhmberg in Dorpat in diagnosing cases by the planting of pus. Thalmann recommends it especially because of its simplicity, durability, sure sterility, transparency, and the certainty of growth. All of the cultures employed in this work grew well on this medium if transplanted from young, not more than twenty-four hour, ascitic agar growths. After seven or eight generations, however, they began to die out and it

was necessary to start them again from ascitic medium. Their longevity, also, was apparently not nearly as great as on ascitic media. In ascitic broth most strains of gonococcus will remain alive for thirty to forty-five days and on ascitic agar almost as long, if the medium is not allowed to dry out.

Technic. — In these experiments on the agglutination of gonococcus with specific immune serum, several methods in the preparation of emulsions of the organism were followed. At first, young (two or three day) ascitic broth cultures were centrifuged and then the deposit emulsified in normal saline solution. This proved to be a very unsatisfactory and unreliable method. Then the organism was cultured for eighteen to twenty hours on ascitic agar slants and the growth washed off and emulsified in normal saline solution. The ascitic agar was titrated to about $+ .3$ to phenolphthalein. Medium of this approximate reaction may easily be obtained in the following way: The agar is prepared in the usual way with meat infusion, one per cent peptone, one-half per cent NaCl and two per cent agar and titrated to $+ 1.5$ to phenolphthalein. To two parts of this agar, which has been thoroughly sterilized, one part of rich sterile ascitic fluid heated to 55° or 60° C. for several days is added and the tube is slanted. It is best to allow the agar to dry out for a few days before adding the ascitic fluid. This fluid is always slightly alkaline to phenolphthalein, generally $-.5$ to $-.8$. Although this is a very simple way of preparing the emulsions, it seemed advisable to obviate any influence which the presence of ascitic fluid in the medium might have on the agglutinations by growing the cultures on agar free from this fluid. Accordingly, agar prepared after the method of Thalmann, already described, was used instead. As has been stated, this medium is quite acid to phenolphthalein, about $+ 1$. As is the case generally in agglutination, gonococcus has been found to agglutinate somewhat higher when grown on alkaline medium than when acid medium is used. There is also a greater tendency to clump spontaneously with the former reaction. The results with this acid medium

are for that reason rather more reliable than with alkaline. In other respects, too, this medium has been found very satisfactory and has been employed in most of the experiments recorded here.

In making cultures for agglutination it is very important to transplant from stock cultures not more than twenty-four hours old. The reason for this will be explained in the discussion of spontaneous agglutination. This phenomenon is one which must be constantly guarded against. Different strains of gonococcus vary greatly in their tendency to clump out spontaneously. Some never do, while others can be prevented from doing so only by the most careful technic. Strains C and F were the worst offenders in this regard. It was found, however, that by adding .2 per cent formaldehyde to the normal saline solution used in making the emulsions, spontaneous clumping could be entirely prevented or reduced to a minimum in every instance.

The emulsions of gonococcus in normal saline solution were always made to as nearly as possible the same density. This standard density was obtained by making a rather thick emulsion and then diluting it and shaking it until it became slightly opalescent. An emulsion of this character was perfectly homogeneous. A microscopical examination showed that the gonococci were well scattered. To .5 cubic centimeter of this preparation .5 cubic centimeter of the diluted serum was added and the tube thoroughly shaken. The tubes containing the mixtures were then placed in the incubator at 37° C. for three hours, and after that in room temperature until the next day. Readings with the aid of a hand lens were made as a rule at the end of one, two, three, and twenty-four hours. Too great care in controlling these agglutinations cannot be taken. A series of tubes containing equal portions of emulsion of each strain and normal saline solution should always be carried along with the others. The greatest dependence was placed on the reading at the end of three hours. If no agglutination could be observed then, any appearing at the end of twenty-four hours was carefully compared with the control to rule out spontaneous clumping.

Immunization of animals. — Rabbits were inoculated with eight different strains of gonococcus. These consisted of the eighteen to twenty-four hour growths from two large ascitic agar slants (the tubes averaging 2.5 centimeters in diameter) emulsified in normal saline solution. These living emulsions were injected into the peritoneal cavity every six or seven days. Although various strains of gonococci differ greatly in toxicity, the animals seldom succumb to this treatment. At first they lose weight, but after four or five inoculations they become thoroughly immunized and begin to gain weight. After the rabbits had received nine to ten inoculations, extending over a period of about eight weeks, the blood was drawn from the carotid.

Agglutination with normal serum. — A review of the meager literature on the subject shows that the agglutination properties of normal serum for gonococci have not been described. In order, however, to control properly the results with immune sera, it is essential that any normal agglutinins which may be present be taken into consideration. All except two strains (D and E) agglutinated in normal rabbit serum in varying dilutions. In the sera of the eight normal rabbits which were tested great diversity in the titer for various strains was encountered. In the following table the results with the sera of rabbits representative of the high and low normal agglutinations are given; and also the results with normal ox and normal horse serum:

TABLE I.
Agglutinations of gonococcus with normal sera.

Sera.	Cultures.						
	A.	B.	C.	G.	H.	I.	J.
Rabbit I.	200	20	100	200	100	200	20
Rabbit II.	10	20	10	50	50	20	20
Ox	4	10	40	0	80
Horse	0	0	0	0	20

The fact that some normal rabbit sera agglutinate certain cultures of gonococcus in dilutions up to at least 1-200 indicates that there may have been here a fruitful source of error in basing the relationship between meningococcus and gonococcus on comparatively low inter-agglutinations with rabbit immune serum. A peculiar feature of these agglutinations with normal rabbit serum is the fact that some strains of gonococcus always show a marked "præ-zone," viz., in dilutions up to 1-10, there may be little or no clumping of the cocci, whereas in higher dilutions it is very marked and appears early. Culture G invariably shows this "præ-zone," while culture C never does. The others may show it with one serum and not with another. So far as I am aware, this phenomenon has never been observed in normal serum with any other bacteria.

Immune rabbit sera. — The following table (II.) is based on a great many observations with living and formalized emulsions of gonococci grown on media with and without ascitic fluid. At times there has appeared some variation in the titer of the different strains with the same serum. This is due, no doubt, to slight changes in the vitality of the cultures and variations in the culture media. The figures in Table II., however, indicate correctly the relationship of the agglutinability of the various strains. In order to find out the titer of a serum for each strain, one should read down a column; to determine the height of agglutination of a single strain with each of the eight sera, one should read across a line. The "homologous" agglutinations run diagonally across the table.

TABLE II.
Agglutinations of gonococcus with immune rabbit sera.

Cultures.	Immune Sera.							
	Rabbit Immu- nized to A.	Rabbit Immu- nized to B.	Rabbit Immu- nized to C.	Rabbit Immu- nized to D. *	Rabbit Immu- nized to F.	Rabbit Immu- nized to G.	Rabbit Immu- nized to H.	Rabbit Immu- nized to I.
A	Homologous. 700,000	20,000	200	800	2,000	800	200	200
B	2,000	Homologous. 20,000	800	800	400	400	500	400
C	80	2,000	Homologous. 5,000	200	1,000	800	1,000	800
D	400	400	10	Homologous. 100	0	200	10	200
E	50	100	400	400	200	800	50	50
F	400	1,000	200	400	Homologous. 3,000	800	200	200
G	200	200	50	400	400	Homologous. 2,000	2,000	1,000
H	200	2,000	20	100	800	2,000	Homologous. 2,000	2,000
I	800	1,000	200	800	2,000	4,000	Homologous. 2,000
J	400	1,000	400	800	2,000	400	400	200

* Received only five inoculations.

In studying this table, the first thing that strikes one is the great variation in the agglutinability of the different strains. Certain ones like A and B are very good agglutinators with their homologous sera, while others like D clump only in low dilutions even with their homologous sera. If only one serum was used in the experiments it might seem doubtful whether or not some of these strains are true gonococci, but by using a number of immune sera, for example B, C, and G, it is possible to find one or more with which each culture has a fairly high titer. This, however, may not be its homologous serum as is shown by culture D, which agglutinates four times as high in sera A and B as it does in its own serum D. In the majority of cases, nevertheless, the highest titer for a culture is with the serum raised by it.

With some of the stronger sera the agglutination of cultures A and B was very rapid. Taking for example serum B, in fifteen minutes with culture B the clumps were plainly visible to the naked eye at 1-200, and with a hand lens at 1-400. In thirty minutes the same was true at 1-400 with the naked eye, and at 1-10,000 with the hand lens. The other cultures, except H, did not show any clumping until the reading at the end of the hour. In the majority of instances the highest reaction of a culture could be determined by the aid of a hand lens at the three-hour reading, especially after the tubes had been in the room temperature for a few minutes. As is generally the case in agglutination the change from incubator to room temperature hastens the reaction. Another point of importance indicated in this table is that some cultures like B and G raise more general agglutinations for all the cultures used, while other cultures, as C, and especially culture A, produce sera which are markedly specific.

The remarkable titer of culture A in its own serum A calls for more than passing notice. As far as I am aware no agglutination as at great dilution has been described for any cocci or diplococci. Durham¹⁴ has described a still more potent serum for typhoid. By inoculating a rabbit for about two years with large quantities of culture he obtained a

serum with a titer of 1-2,000,000. As a rule, however, the greatest potency he obtained in sera immune to bacilli was 1-500,000. Walker¹⁵ and Tavel have also obtained an anti-typhoid serum with a very high agglutinative titer. After immunizing a horse for two years with large quantities of culture the serum agglutinated a particular strain of *B. typhosus* at the dilution 1-2,800,000. This test of culture A with serum A has been made repeatedly and there can be no question of its accuracy. The rapidity of the reaction is shown in Table III. The tests were made with cultures grown on ascitic agar and also on Thalmann's medium, which contains no ascitic fluid. In the controls normal saline solution was substituted for serum. There was not the slightest indication of spontaneous agglutination or precipitation in these control tubes.

TABLE III.
Rate of the agglutination of culture A in serum A.

Culture.	Serum.	Dilutions of Serum.																Control.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																									
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		1 hr.	2 hrs.	3 hrs.	24 hrs.	1 hr.	2 hrs.	3 hrs.	24 hrs.	1 hr.	2 hrs.	3 hrs.	24 hrs.	1 hr.	2 hrs.	3 hrs.	24 hrs.		1 hr.	2 hrs.	3 hrs.	24 hrs.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
A (grown on Thal- mann's media with- out ascitic fluid)	A.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ L = clumps visible with the aid of a hand lens.

It is rather surprising to find that a culture grown on ascitic agar agglutinates more rapidly and higher than one grown on agar without ascitic fluid, as the presence of such fluid generally has an inhibitory influence. This latter is the case with strains G, H, I, and J, as they agglutinate higher with serum A when grown on agar without ascitic fluid. With the other sera, also, the use of ascitic fluid seems to exercise a slight inhibiting influence.

As has already been indicated, a "præ-zone," in which there is no agglutination, is frequently encountered in the normal sera of the rabbit, especially when certain cultures are used. This same phenomenon is also often met with in testing sera immune to gonococcus. The same cultures, too, seem to show a marked tendency in this direction. With serum H, the reading for culture G at one hour was — at 1-10, + at 1-100, + at 1-200, + L at 1-500, and — at 1-2,000. A similar result was obtained with sera D and I, the "præ-zone" extending to 1-200. The number of inoculations the animal has received seems to have nothing to do with this phenomenon, for a rabbit which had received only one inoculation with culture B showed a marked "præ-zone" for cultures A, B, D, and E at 1-10, whereas in the serum of an animal which had received ten inoculations there was no such zone. With the sera of highly immunized goats and sheep, cultures B and A agglutinated poorly or not at all in the low dilutions, whereas C did so. Culture C never showed a "præ-zone" with any serum, normal or immune. This phenomenon, with gonococcus at least, seems to be a purely physical one, and the term "pro-agglutinoids" with its biological implication would be misapplied.

As has been mentioned, the cultures used in the inoculation of these rabbits were grown on ascitic agar. To forestall any possible criticism of the use of ascitic fluid in the media, rabbits were inoculated with cultures C and G grown on Thalmann's plain agar. Except with culture C there was no noteworthy difference in the sera raised in the two ways. Culture C, however, agglutinated very much better in serum produced with C cultures grown on media without ascitic

fluid. The highest titer obtained, using the first method, was 1-5,000, whereas with the second technic the agglutination was very rapid and complete up to 1-50,000. It is possible that this was due in a measure to the idiosyncrasy of the rabbit.

In producing a serum for therapeutic purposes, goats and rams have been largely used. The agglutinative value of their sera is never as high as in rabbits, in all probability because it is not practical to give them as large proportional inoculations. The highest titer with the goat immune serum for culture A was 1-2,000, and of that with ram serum, 1-10,000.

The following experiment is designed to show the effect of the number of inoculations on the titer for each strain. In this table (IV.) are compared the agglutination with normal rabbit serum, with rabbit immune serum after one inoculation with culture B, after five inoculations and after nine inoculations of the same organism. The serum of the first two columns is from the same rabbit, and that of the last two from another rabbit.

TABLE IV.
The amount of agglutinin which is produced after one, five, and nine inoculations of culture B.

Sera.	Cultures.									
	A.	B.	C.	D.	E.	F.	G.	H.	I.	J.
Normal rabbit serum * ..	10	20	20	0	0	50	50	50	20	20
Same rabbit after one inoculation of B.....	1,000	600	400	400	400	50	0	0	10	20
Another rabbit after five inoculations of B.....	5,000	5,000	800	800	200	800	200	400	800	400
Same rabbit as preceding after nine inoculations of B.....	20,000	20,000	2,000	400	100	1,000	200	2,000	1,000	1,000

* The lowest test was made at 1-10.

A striking feature of this table is the rapidity with which agglutinins are produced for cultures A and B and to a somewhat less extent for C and D. On the other hand, for

cultures G, H, and I there is an actual loss after the first inoculation of even the agglutinins in the normal serum. With culture G, the maximum titer appears after five inoculations, and with D, after one inoculation. Further inoculations cause no increase in the height of agglutination for these cultures and for culture E even bring about a decrease in agglutination. Conditions such as these indicate the probability that we are dealing here with at least two sets of agglutinins. The one, specific or major, which are produced with great rapidity and agglutinate cultures A and B in high dilutions, and the other, minor or group agglutinins, which cause the clumping of the other strains. In order to verify this supposition we must direct our attention to absorption experiments.

ABSORPTION EXPERIMENTS. Technic. — These absorptions, with one exception, were carried out with cultures grown on agar without ascitic fluid. All sera were diluted to 1-50 and then ten cubic centimeters of this dilution was absorbed with the growth from a definite number of cultures for each serum. For example, ten cubic centimeters of diluted G serum (which is equivalent to 1/5 cubic centimeter of undiluted) was always absorbed with the growth from six cultures. After two hours at incubator temperature the mixture of serum and gonococcus was centrifugalized for seven to ten minutes. This proved sufficient in nearly all cases to clear the serum. Control experiments, which were carried out with unabsorbed serum, seemed to show that the physical conditions to which the serum is subjected in this technic cause little if any destruction of agglutinin.

In the following table (V.) the results of the absorption of serum B with cultures A, C, and D are given. The lowest test of absorbed serum was necessarily made at 1-100.

TABLE V.

Serum B absorbed with cultures A, C, and D. The growth from six tubes used in the absorptions with cultures C and D.

Cultures.	Serum B before Absorption.	Serum B after Absorption with Culture A.*	Serum B after Absorption with Culture C.	Serum B after Absorption with Culture D.
A	20,000	1,000	20,000	20,000
B	20,000	5,000	20,000	20,000
C	2,000	400	0	2,000
D	400	0	0	0
E	100	Not tested.	0	0
F	1,000	Not tested.	1,000	1,000
G	200	100	200	200
H	2,000	1,000	2,000	2,000
I	1,000	1,000	1,000	1,000
J	1,000	Not tested.	1,000	1,000

* The growth from five ascitic agar tubes was used in this absorption.

From these experiments it appears that there are at least four different groups of agglutinin in this serum. First, the agglutinin for cultures D and E. This is evidently different from that for culture C, for although culture D absorbs all the agglutinin for culture E, this culture does not take out any of the agglutinin for C. Second, that for culture C. When this is removed from the serum by absorption the agglutinins for A, B, F, G, H, I, and J are not reduced in amount, although those for D and E are all absorbed. Third, the agglutinin for cultures F to G, which is not removed by absorption with C and D and which the following tables show is quite distinct from the agglutinins for A and B. Fourth, the major or specific agglutinins for B, which are absorbed in part by culture A, together with all the other group agglutinins except those for I. This fact shows that cultures A and B are practically identical or very closely related organisms. For further light on the

inter-relationship of these cultures we must pass on to absorption of other sera.

TABLE VI.

Serum C absorbed with cultures A, G, and I. The growth from three tubes used in each absorption.

Cultures.	Serum C before Absorption.	Serum C after Absorption with Culture A.	Serum C after Absorption with Culture G.	Serum C after Absorption with Culture I.
A	200	0	100	100
B	800	0	400	400
C	5,000	5,000	5,000	5,000
E	400	0	0	0
F	200	0	200	200
I	200	200	200	0
J	400	0	200	200

With serum C, then, we find that absorption with cultures A, G, and I have absolutely no effect on the agglutinins for culture C, viz., the major agglutinins. These cultures, accordingly, are specifically different from C as far as agglutination is concerned. In the absorption of this serum with culture A the agglutinins for cultures B, E, F, and J are also removed, and yet in absorbing with the same amount of culture I only the agglutinins for E are taken out, although the titer of these two cultures is the same in unabsorbed serum C. This shows that one cannot judge of the absorbing qualities of a culture from the height of its agglutination; for example, a culture of gonococcus may have a high titer in a serum produced by another strain, and yet may absorb little more than its own agglutinins. Referring back to Table V. we find this point illustrated in another way. Although culture C agglutinates well in serum B, it absorbs only the agglutinins for cultures D and E. The agglutinins for these two cultures, by the way, are removed far more readily in all the experiments than those for the other strains.

Even culture G, which agglutinates no higher than 1-50 with C serum, removes from it the agglutinin for E.

The larger part of the absorption experiments were carried out with serum G, as the agglutinations by this serum of all the various strains were fairly high.

TABLE VII.

Serum G absorbed with cultures A, C, E, F, H, I, and J. The growth from six tubes was used in each absorption.

Cultures.	Serum G before Absorption.	Serum G after Absorption with Culture A.	Serum G after Absorption with Culture C.	Serum G after Absorption with Culture E.	Serum G after Absorption with Culture F.	Serum G after Absorption with Culture H.	Serum G after Absorption with Culture I.	Serum G after Absorption with Culture J.
A	800	0	800	800	800	0	100	800
B	400	400	400	400	100	200	100	0
C	800	0	0	0	0	0	0	0
D	200	0	100	0	200	0	0	0
E	800	0	400	0	0	0	0	200
F	800	0	400	400	0	0	0	800
G ...	2,000	2,000	2,000	2,000	2,000	800	800	2,000
H	2,000	2,000	2,000	2,000	1,000	800	800	2,000
I	2,000	2,000	2,000	2,000	2,000	800	800	2,000
J	400	400	200	100	100	0

Of the seven strains tested, only two, H and I, remove in part the agglutinins for G, viz., the specific or major agglutinins. These two cultures, too, act on all the minor or group agglutinins. We may conclude, then, that cultures G, H, and I are identical or very closely related. The cultures A and B are not agglutinated by identical minor agglutinins, although these cultures are clumped by the same major agglutinins. Culture A absorbs none of the agglutinins for culture B, and, on the other hand, culture J removes all the agglutinin for B and none for A. In none of these experiments has culture C shown the slightest agglutination relationship to the group A, B on the one side, nor to group G, H, I on the other.

Sufficient data have certainly been presented to indicate the confusing nature of these agglutinin relationships as revealed by absorption experiments; and also possibly enough to weaken our confidence in the dictum that specific relationship should be based on the absorption of major agglutinins from a serum. If we accept this principle, the conclusion is unavoidable that the term *gonococcus* covers a large number of organisms which differ among themselves as greatly as the different types of the dysentery bacillus or the streptococcus group. Unfortunately, the enzymatic activities of *gonococcus* on sugars is practically nothing, and so we cannot make use of such differences in dividing the several cultures into groups. Basing our conclusions, however, entirely on the results of the absorption experiments, we find three types which agglutinate wholly independently of one another.

Type I. Represented by cultures A and B. — They are very good agglutinators in their own serum and also give rise to agglutinin quickly and abundantly. These are induced to grow on ordinary agar with difficulty, and die out much more quickly than the others under most favorable circumstances. Culture A is much more toxic for laboratory animals than any other which was used. Culture B is also decidedly toxic.

Type II. Represented by cultures G, H, and I. — These are not agglutinated by the same agglutinins as Type I. or Type III. They agglutinate and raise agglutinins moderately well. G and H grow quite readily on agar without ascitic fluid; I, much less so. Moderately toxic for laboratory animals.

Type III. Represented by culture C. — This is not agglutinated by the same agglutinins as Type I. or Type II. It agglutinates fairly well and grows very readily on agar without ascitic fluid. Slightly toxic for laboratory animals, less than Type II.

By describing these types of gonococci it is not implied, by any means, that all cultures of gonococci may be classified under them. There are all manner of intermediate

forms and variations. Cultures D and E cannot be placed under any one of these types, for although they are not cultivated easily on agar without ascitic fluid, and in that respect resemble Type I., they are poor agglutinators and are not clumped by the same agglutinins as that type. Again, cultures F and J bear some agglutinative relationship to Type I., but are not especially toxic.

Gonococcus is a purely parasitic organism which may attack many different parts of the host. It is natural to suppose that the diverse environments to which this organism is subjected must call forth adaptive changes; actively as regards its growth, and passively as regards its reaction to sera. Similar variations have long been known in streptococcus, and it is still a moot question whether they are of major or minor importance. As regards *B. dysenteriae* there are a number of types which differ fundamentally in their enzymatic activities and the agglutinin and immune bodies which they produce. That cultures of gonococci differ greatly as regards the ease with which they may be cultivated outside the body, and the agglutinins to which they give rise, is certainly true. Whether these differences are sufficiently great to make it necessary to take them into consideration in serum-therapy is the point of practical interest. This will be discussed from other points of view in subsequent articles. Suffice it to say at this point that the evidence seems to warrant the employment of cultures representing each of the several main types of gonococcus in the production of a serum for therapeutic purposes.

Effect of the passage through animals on the agglutinability of a culture of gonococcus. — It has been shown for a number of bacteria that their passage through laboratory animals causes a marked decrease in their agglutinability. This has been found to be true for *B. typhosus* by Buxton and Vaughan;¹⁶ and for streptococcus by Neufeld¹⁷ and Aronson.¹⁸

In the following table (VIII.) the titer of culture A in serum A is indicated after a number of passages through the peritoneal cavity of guinea-pigs.

TABLE VIII.

Effect on the agglutinability of a culture of gonococcus of passage through a series of guinea-pigs.

Serum.	Cultures.					
	Culture A.	Culture A passed through One Pig.	Culture A passed through Three Pigs.	Culture A passed through Six Pigs.	Culture A passed through Eight Pigs.	Culture A passed through Ten Pigs.
A	700,000	5,000	2,000	5,000	5,000	2,000

By the first passage through the peritoneal cavity of a guinea-pig there was a very marked decrease in the agglutinability of culture A in the serum raised by it. This amounted to one hundred and forty times. After the third passage there was another slight reduction, but there was no further decrease even after the passage through ten pigs. Exactly the same results were obtained by Buxton and Vaughan¹⁶ with *B. typhosus*.

Agglutination of meningococcus in anti-gonococcic serum.—Bruckner and Cristéanu found that the serum of the horse, which they immunized to several strains of gonococcus grown on coagulated serum, agglutinated meningococcus in as high dilutions as it did gonococcus. The titer for gonococcus was 1–2,000 and that for meningococcus exactly the same. Vannod, also, found an inter-agglutination relationship between gonococcus and meningococcus. His anti-gonococcic serum with its titer of 1–1,000 for gonococcus, agglutinated meningococcus as high as 1–300. The latter investigator used a nucleo-proteid of gonococcus in immunizing his rabbits. Through the kindness of Dr. Elser and Dr. Huntoon, I obtained a culture of meningococcus, which, according to their tests, agglutinates very well in anti-meningococcic serum. With potent sera its titer was 1–7,000. In the following table (IX.) its titers with the eight gonococcus sera are given. For comparison, the agglutinative value of the homologous strain of gonococcus in its own serum is added.

TABLE IX.

Agglutination of meningococcus in the various anti-gonococcic sera.

Cultures.	Sera.							
	A.	B.	C.	D.	F.	G.	H.	I.
<i>Gonococcus</i> (Hermol.) (Gibbs)	700,000	20,000	5,000	100	3,000	2,000	2,000	2,000
<i>Meningococcus</i>	50	100	10	500	50	50	50	10

With the exception of serum D, meningococcus agglutinates only in very low dilutions of these anti-gonococcic sera, in fact little higher than they do in normal rabbit serum. The rabbit immunized to culture D had received about half as many inoculations as the others, and its serum was possibly for that reason less specific than that of the more highly immunized animals. The results, as a whole, certainly show very little agglutinative relationship between the gonococci and the meningococcus employed in these tests.

Spontaneous agglutination. — As has been stated, some strains of gonococcus clump spontaneously under certain conditions, due, probably, to their having been cultivated for many generations in serum media. In a study of their agglutination with immune sera this must be continually borne in mind and guarded against. A number of factors which tend to bring about spontaneous agglutination may be mentioned. It has been found that if the culture used in the agglutination test has been planted from a culture several days old there is a far greater tendency to clump out spontaneously than if one twenty-four hours old is used. Another predisposing factor is too great moisture of the agar. It is better to let the media dry out a little before making cultures for agglutination tests. The addition of ascitic fluid to the media, especially if this fluid is rich in albumen, may also cause more or less rapid spontaneous agglutination with some cultures. If ascitic agar is used it is best to choose an ascitic fluid which is thin and poor in albumen. As has

already been mentioned, however, the results in this paper are based almost entirely on tests made with cultures grown on agar to which no ascitic fluid has been added. As was indicated in the first part of this paper, a very useful expedient in preventing this pseudo-agglutination is to make the emulsions of the organism in normal saline solution to which .2 per cent formaldehyde has been added. If these precautions are observed, spontaneous clumping may be entirely eliminated.

A peculiar fact in connection with this phenomenon is the inhibitory influence which serum may have on it. It might be expected that spontaneous agglutination would be as marked in all dilutions of immune or normal serum as it is in the normal saline solution control. Such, however, is not the case. In the lower dilutions of the serum the clumping is as rapid or more so than in the control, depending upon the amount of agglutinin. But in the high dilutions there may be no agglutination at all. In absorbed serum, also, if all the agglutinins for one of these cultures, which have a tendency to clump spontaneously, have been extracted, the culture will not clump at all in the lower dilutions, or far less than the control. It is evident that dilutions of serum in which there are no agglutinins for a culture of gonococcus, as is the case when it is highly diluted or absorbed, change the physical conditions of the solution in such a way as to prevent the cocci from clumping together spontaneously.

PRECIPITINS. — With the exception of the first announcement by the writer of specific precipitins in anti-gonococcic serum, there is only one article bearing on this subject, and that is a very brief note by Bruckner and Cristéanu⁴ to which reference has already been made. These investigators prepared the precipitinogen by macerating cultures grown on serum agar in .15 per cent solution of NaOH, and then filtering through porcelain. They give no details, but merely state that a filtrate prepared in this way gives an abundant precipitation with anti-gonococcic serum, but none with normal serum. They obtained the same precipitation,

however, with gonococcic immune serum, if an extract of meningococcus prepared in the same way was used. A maceration of gonococci in normal saline solution for twenty-four to forty-eight hours produced only a feeble precipitinogen.

It was found very early in the experiments recorded here, that if ascitic agar cultures were used in producing the immune serum then the precipitinogen must be prepared from cultures grown in media without a trace of ascitic fluid. Even the serum from animals inoculated with the growth scraped off from ascitic agar precipitates heavily broth which contains a very small amount of ascitic fluid or salt solution with which the surface of an ascitic agar tube has been rinsed. In order to overcome this difficulty a series of precipitation tests were carried out with filtrates of cultures grown in plain broth prepared according to Thalmann's method. Cultures of gonococcus were selected which grow well in this medium. These, and also cultures of meningococcus, *M. catarrhalis* and staphylococcus, were planted in small flasks of this broth, titrating + .8 to phenolphthalein, and incubated for five weeks, the cotton plugs being paraffined to prevent evaporation. The filtrates were obtained by passing the broth cultures through a layer of sterile talc on filter paper. They were perfectly clear. The experiments were controlled with sterile broth of the same character. In the following table (X.) the titers with five anti-gonococcic sera are given.

In making the tests .5 cubic centimeter of the filtrate was added to .5 cubic centimeter of the serum dilution and the tubes were then briskly shaken. Readings were taken at the end of one, four, and twenty-four hours. The tubes were placed in the incubator at 37° C. for four hours, then in room temperature. The highest dilution in which flocculi could be distinctly seen at the end of twenty-four hours with the aid of a hand lens is given in the table.

TABLE X.
Precipitations with filtrates of broth cultures.

Filtrates.	Sera.					
	Normal Rabbit.	Rabbit A.	Rabbit B.	Rabbit C.	Rabbit D.	Rabbit G.
C	0	200	100	50	20	50
D	Cloud at 4.	350 ¹	50	20	200	50
F	Cloud at 4.	200	100	100	100	20
Meningococcus .	Cloud at 10.	50 ²	20	4	0	4
Catarrhalis	0	0	0	0	0	0
Staphylococcus..	0	0	0	0	0	0
Control	0	0	0	0	0

¹ Trace in twenty-four hours at 500.

² Trace in twenty-four hours at 150.

With normal rabbit serum there was practically no reaction with any of the filtrates. A slight cloud appeared in four hours with filtrates D and F at a dilution of 1-4 and with meningococcus up to 1-10. No flocculi, however, were visible, nor any precipitate. Of the immune sera, A caused much the highest precipitation, in like manner as it has been shown to contain the greatest amount of agglutinin for culture A at least. Although culture C agglutinated much higher in its own serum C than in serum A, yet it precipitated four times as high in the latter. Filtrate D precipitated seven times as high in serum A as in serum B, and yet its agglutination titer was the same in each. There seems to be, accordingly, no relation between the agglutinating and precipitating values of these gonococcal anti-sera and cultures. Culture D actually precipitates twice as high as it agglutinates in its own serum. All of the immune sera, except D, contain some precipitins for meningococcus, and yet in serum D we have found the greatest amount of agglutinin for this organism. Bruckner and his

colleagues found that there was as much precipitin for meningococcus in the serum of an animal immunized to gonococcus as for gonococcus. The experiments recorded here do not lead one to that conclusion. In the five sera which were tested, the meningococcus filtrate precipitated in not higher than one-fifth the optimum titer of the gonococcus filtrates, and generally only in one-tenth or less. The results of Bruckner and Cristéanu may be explained probably on the ground that they used serum agar cultures in immunizing their animals and extracts from serum agar cultures in testing for precipitins. As has been stated, the precipitins raised for the serum in the media, when this technic is used, entirely mask the precipitins for the cultures themselves. My results, certainly, seem to indicate some relationship between gonococcus and meningococcus, but not as close a one as is implied in the results of the above-mentioned investigators. For *M. catarrhalis* there were no precipitins in the various anti-gonococcic sera. As it is impossible with most cultures, at least of this organism, to obtain an emulsion which may be employed in agglutination tests, precipitation tests are of especial value in showing that *M. catarrhalis* and gonococcus belong to groups which are not related. A filtrate of staphylococcus culture also, as one would expect, is not precipitated in an anti-gonococcic serum.

The following table (XI.) has been inserted to show the rate at which the various filtrates are precipitated by serum A :

TABLE XI.
Precipitations of filtrates of broth cultures with serum A.

Filtrates.	Serum.	4			10			20			50			100			150		
		1 hr.	4 hrs.	24 hrs.	1 hr.	4 hrs.	24 hrs.	1 hr.	4 hrs.	24 hrs.	1 hr.	4 hrs.	24 hrs.	1 hr.	4 hrs.	24 hrs.	1 hr.	4 hrs.	24 hrs.
C.....	A	+ L	+	+++	-	+	+++	-	+	+++	-	+	+	-	-	+ L	-	-	+ L
D.....	A	+ L	+	+++	+ L	+	+++	+ L	+	+++	+ L	+	+	Cl.	+ L	+	-	+ L	+
F.....	A	+ L	+	+++	Cl.	+	+++	-	+ L	+	-	+	d.	-	-	d.	-	-	d.
Meningococcus..	A	Cl.	+	+++	Cl.	+	+++	-	+ L	+	-	+	+ L	-	-	+ L	-	-	+ L
Catarrhalis	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus ..	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Filtrates.		200			250			300			350			400			500		
C.....	A	-	-	+ L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D.....	A	-	+ L	+	-	+ L	+	-	Cl.	+	-	Cl.	+ L	-	-	Trace.	-	-	Trace.
F.....	A	-	-	d.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ L = flocculi visible with aid of hand lens.

Cl. = cloud.

d.

+ L = slight deposit in tube, reaction less than +.

It seemed advisable to control these experiments with filtrates of broth cultures by others in which a gonococcic precipitinogen was obtained in some other way. Accordingly a technic devised by Wadsworth¹⁹ for obtaining extracts from pneumococcus was employed. This consists in shaking the cells in seventeen per cent NaCl solution in distilled water and then diluting to the density of the normal solution. The cultures were grown on Thalmann's agar without ascitic fluid and extracts obtained by this method. They were then filtered through sterile talc, the filtrates being perfectly clear and colorless. For a control, a tube of sterile agar was washed off with the concentrated salt solution, and the diluted wash filtered through talc. If a sterile ascitic agar tube is treated in this way the addition to the filtrate of serum from a rabbit immunized to ascitic agar cultures will cause a heavy precipitation. In Table XII. the results of these experiments are shown.

TABLE XII.
Precipitations with seventeen per cent NaCl extracts.

Filtrates.	Sera.		
	Serum of Rabbit A.	Serum of Rabbit C.	Serum of Rabbit G.
A.....	100	20	50
C.....	50	20	20
G.....	50	20	20
Catarrhalis	4	0	0
Control.....	4	0	0

The results on the whole are not as satisfactory as when broth filtrates are used, since the precipitation takes place more slowly and in lower dilutions. It was not possible to obtain a clear filtrate of meningococcus by this method, but, as before, there is little or no precipitation of the extract of *M. catarrhalis*. We find again that serum A causes the strongest precipitation. Serum G presented a marked

"præ-zone" at the dilution of 1-4. It will be remembered that this serum showed a similar retardation of agglutination in low dilutions.

CONCLUSIONS.

Rabbits and other laboratory animals, when inoculated with cultures of gonococcus, produce specific agglutinins and precipitins.

Normal rabbit sera contain a varying amount of agglutinin for gonococcus.

Strains of gonococci differ greatly in the titer of their agglutination with various gonococcic immune sera.

After one inoculation a large amount of agglutinin was produced for some cultures, but none for others. Further inoculations cause an increase in the titer of the agglutination of certain strains, but a drop in that of others.

Absorption experiments indicate that an anti-gonococcic serum may contain in addition to the specific homologous agglutinin several groups of agglutinin which act on different cultures quite independently of one another. At least three groups were found whose major or specific agglutinins are not removed by inter-absorptions. This indicates that as far as agglutination is concerned there are specific differences between these groups. The family gonococcus is, accordingly, heterogeneous rather than homogeneous. In making a serum for therapeutic purposes this fact should be borne in mind.

The passage of a culture of gonococcus through a guinea-pig caused a very marked drop in the agglutinability.

With the exception of one serum, meningococcus agglutinated only in low dilutions of the anti-gonococcic sera.

Anti-gonococcic sera contain specific precipitins for gonococcus.

There appeared to be no relation between the precipitating and the agglutinating properties of an anti-gonococcic serum for a culture of gonococcus.

Anti-gonococcic sera contain, as a rule, some precipitins

for meningococcus, but none for *M. catarrhalis* or staphylococcus.

There is evidence of a relationship between gonococcus and meningococcus, but not of as close a one as has been described by some investigators.

[My thanks are due to my colleagues, Dr. Elser and Dr. Huntoon of Cornell Medical College, and also to Dr. R. J. Wilson of the New York Health Department for the majority of the cultures.]

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THE DEGREE AND DURATION OF PASSIVE IMMUNITY TO
DIPHThERIA TOXIN TRANSMITTED BY IMMUNIZED
FEMALE GUINEA-PIGS TO THEIR IMMEDIATE OFFSPRING.*

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In a preliminary paper published several years ago, the writer called attention to the fact that certain female guinea-pigs under observation gave birth to young which had more than average resistance to diphtheria toxin.¹ This increased resistance was observed in all litters until the death of the mother, and was nearly constant in amount for any given mother. At the time any preliminary treatment was ruled out as a factor because of mothers treated alike some gave birth to normally susceptible young. A continuation of the observations on a relatively large number of animals soon showed that the inference drawn was half true and half false. The conclusions finally reached were that the manifestation of increased resistance in the young is due to preliminary treatment of the mother with toxin-antitoxin mixtures, but the degree to which the mother reacts to treatment — that is, the degree of passive immunity transmitted to the young — is probably an individual or family factor. It was also found that most of the phenomena observed were easily brought under the principles laid down by Ehrlich in his celebrated researches dating from 1892. There was, however, an important untouched residuum of data brought to light which the writer considered of sufficient importance to justify the publication of his experiments at this time. Before detailing these a brief review of the literature is necessary.

In his first paper² Ehrlich showed:

1. That female mice, immunized to ricin, abrin, and robin gave birth to young which, four weeks after birth,

* Received for publication April 23, 1907.

possessed distinct resistance. This was lost at beginning of third month.

2. That the grandchildren possessed no increased resistance or immunity, and
3. That immune fathers do not transmit any immunity to their offspring.
4. That in mice lactation plays an important part in the transmission of immunity to offspring, and that normal offspring may gain a considerable degree of immunity by being nursed by immune mothers.

Ehrlich and Hübener³ in a later paper extended these studies to tetanus, and showed that

1. Immune mothers (guinea-pigs and mice) transmit tetanus immunity to young. Fathers do not.
2. Immunity in the young disappears at the end of the second, surely after the third month.

A paper by Wernicke,⁴ published in 1895, had completely escaped the writer's notice when his first paper was written. This was probably due to the mode of publication. This paper was the only one bearing immediately on the subject of this communication. Wernicke's guinea-pigs were treated originally with toxin, then with antitoxin, and repeatedly treated with toxin subsequently. He used cultures of undetermined toxicity for immunization. He found that the treatment of female guinea-pigs between litters increased the resistance of successive litters, that the father does not transmit immunity, that there is no residual immunity in grandchildren, and that the immunity of offspring is still present in the third month. His experiment to determine the influence of lactation on immunity in the guinea-pig was partly successful, but its relation to immunity was not cleared up.

Vaillard⁵ experimented not only with tetanus, but also with anthrax, cholera and *Vibrio Metschnikovi* (*V. avicide*). In his experiments the immunized father in no case influenced the young, but the treated mother always transmitted some resistance which in one case lasted through four litters.

Tetanus immunity disappeared in third or fourth month. His nursing experiment shows that the process does not confer any immunity, nor detract from it, in guinea-pigs or rabbits. If these results should be confirmed they would establish a fundamental difference between mice, on the one hand, and rabbits and guinea-pigs on the other, as regards the influence of lactation. Vaillard also noted *one* case of resistance in the second generation. He finally states that immunity is not the same in all individuals of the same litter.

Remlinger's results⁶ are the same as Vaillard's as regards the father's negative influence, and that of nursing. He also emphasizes the short and fugitive duration of the passive immunity conferred.

Bulloch⁷ approached the same problems by the use of rabbits immunized with blood corpuscles of the ox, and, therefore, accumulating in their blood specific hemolysins. The blood serum of the young of three successive litters contained the specific hemolysins. He, in conformity with the earlier observers cited above, found that the male transmits no immunity to the young.

Dieudonné⁸ experimenting on guinea-pigs with agglutinins, finds the same laws governing the transmission of the latter as those governing toxin immunity.

After most of the data and all the facts here to be presented had been brought together, Anderson⁹ published a short paper dealing with the same subjects. The main features of this paper are the inferences that the treatment of the mothers is responsible for the increased resistance of the offspring, and that the single injection of toxins alone does not lead to any increased resistance of the offspring. With these the writer fully agrees, having reached them, as stated above, before Anderson's paper had appeared.

Two papers¹⁰ in which a claim is made that the father transmits an acquired immunity need only be mentioned here, for all observers since 1893 have obtained uniformly opposite results. Ehrlich has also shown the inadequate character of the experiments upon which the conclusions were based.

I purposely omit reference to those papers in which fowls and their ova were the subject of similar experimentation.

METHOD OF TESTING INCREASE IN RESISTANCE TO DIPHTHERIA TOXIN.

The method used throughout is the familiar one of injecting toxin-antitoxin mixtures, a process carried out so extensively to-day in the routine testing of the strength of diphtheria antitoxin. For the benefit of those who are interested in the broader problem of heredity from the general biological standpoint, and who are not familiar with the procedure for testing antitoxins, the method will be briefly described.

The toxin unit employed is contained in that amount of filtered culture fluid which, when injected subcutaneously into a guinea-pig of approximately two hundred and fifty grams in weight is just sufficient to prove fatal. This minimum fatal dose kills in three to four days. When death ensues sooner than this, provided the injecting fluid did not enter the peritoneal cavity, the dose is too large. This is the only measure available at present.

The antitoxic unit is the standard unit for measuring the strength of diphtheria antitoxin. This unit, contained in a given quantity of serum, will neutralize completely a given amount of diphtheria toxin. This complete neutralization is determined by injecting the mixture under the skin of guinea-pigs. If no local or general disturbance follows, the toxin is considered neutralized.

Toxins prepared at different times or from different cultures of the diphtheria bacillus usually differ slightly from one another in strength as well as affinity for the antitoxin, so that the antitoxic unit does not necessarily completely neutralize the same amount of each kind. This amount has to be determined by actual tests for each lot of toxin prepared, and is designated the L_0 dose. By adding to this dose small amounts of toxin, a mixture will eventually be obtained which contains enough surplus toxin to be just fatal to the guinea-pigs into which it is injected. The dose is called the $L+$ dose. In the testing of diphtheria antitoxin the $L+$ dose is used instead of the L_0 dose, since the former offers opportunities for finer discriminations. The L_0 and $L+$ doses are always multiples of the minimum fatal dose. The actual number of minimum fatal doses contained in the L_0 and the $L+$ dose is not the same in different lots of toxin. In the toxins used by me the $L+$ dose is about thirty-eight times the minimum fatal dose.

In the experiments to be described three lots of toxin have been used, on 13, 14, and 15. The $L+$ dose of No. 13 was .29 cubic centimeter

and gradually rose to .30 cubic centimeter. This means that if serum containing one antitoxic unit was mixed with .29 cubic centimeter or .30 cubic centimeter of toxin, and the mixture injected into the subcutis of a guinea-pig weighing two hundred and fifty to two hundred and eighty grams, it would die in three to four days. The second toxin, No. 14, had a lower L+ dose, .24 cubic centimeter, and later .25 cubic centimeter. The third lot, No. 15, had an L+ dose of .21 cubic centimeter.

The increased resistance of any family of young guinea-pigs was demonstrated by the increased amount of diphtheria toxin which was required when mixed with one unit of antitoxin to kill the guinea-pigs. Thus, with some families the L+ dose instead of being .24 to .25 cubic centimeter was .40 to 60 cubic centimeter toxin. This increased resistance was also shown by a larger minimum fatal dose. It might be asked why not simplify the procedure of testing the immunity by injecting simply the toxin and noting the differences in the minimum fatal doses? This would have been on the whole the better way, but our tests were made in the routine examination of antitoxins and utilized to increase the accuracy of such tests. We had thus two objects in view, both of which were promoted by using the method prescribed.

The result of the injection of antitoxin alone under the skin in doses of five or six cubic centimeters may be rapid death in case the guinea-pig has been made sensitive by previous treatment or inheritance, or else negative. In the cases given in the table there was no appreciable reaction. The injection of toxin alone produces edema, induration, sloughing, and ulceration at the site of injection in the order named. The process may stop at any stage and go no further. The same series of changes may follow an injection of toxin-antitoxin mixtures, the amount of local injury produced depending on the amount of surplus or unsaturated toxin in the mixture. It will be noticed that many of the breeding animals in Table I. developed no local or general lesions, that is, none were noticeable on the third day after the injection of the toxin-antitoxin mixtures.

THE TRANSMISSION OF PASSIVE IMMUNITY BY FEMALE GUINEA-PIGS TREATED WITH DIPHTHERIA TOXIN-ANTITOXIN MIXTURES.

In order to present in their relation to one another the various data compiled during the past four years before taking up a discussion of them from different points of view, the following general table has been drawn up. It gives the immediate source of the mother, whether she was bred in the laboratory or obtained from outside, the resistance of the mother when tested early in life — when about thirty days old — the mode of treatment, whether with toxin or

antitoxin alone or with a mixture of both, the lesion produced, if any, and the resistance of the offspring. The resistance of the mother or offspring as given in the table was determined either upon the animal itself or upon one or more of the same litter, on the assumption that all individuals of the same litter possess the same degree of resistance when tested at the same age, an assumption to which the writer has never encountered an exception. This increased resistance is expressed both in this table and elsewhere in the form of a fraction, the denominator of which represents the normal resistance or L+ dose. The sign + after the numerator means that the resistance is actually greater, and that the L+ dose for that animal or litter had not been reached. The sign ++ means that the resistance is much higher, and that the dose quoted resulted in no lesion at all, or only in a trace. For the convenience of the reader, the fraction is reduced to a decimal which follows it in parenthesis. The source of the mother guinea-pig is given either in the number of the grandmother, when bred by us, or in the form of letters, each letter representing a different leader, excepting A, which refers to our own earlier stock.

The preliminary treatment of the breeding pigs with exceptions given below was a single injection of toxin, or antitoxin (five to six cubic centimeters), or a mixture of both. The amount of antitoxin injected in the latter was one unit. The amount of toxin injected with the antitoxin in the L+ dose was roughly thirty-eight minimum fatal doses. The guinea-pigs were placed in the breeding pens when over five hundred grams in weight. This occurred from three to four months after birth, or from two to three months after the inoculation. In no case were any animals here tabulated treated shortly before or during pregnancy, nor was the original immunity due to the first injection, reinforced by any subsequent treatment, excepting in the case of several guinea-pigs treated with antitoxin only, which received two or three doses some weeks apart, and one other animal (3066) which received toxin and then antitoxin a month later.

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TABLE I.

Mother, Data Concerning.					Resistance of Offspring.
Designation.	Source: the Number is that of Grandmother.	Resistance.	Preliminary Treatment.	Result.	
2602..	A	—	None.	—	Normal.
2870..	"	—	Toxin-antitoxin.	Ulcer.	"
2872..	"	—	" "	No lesion.	35/30 (1.166)
2894..	B	—	None.	—	Normal.
2898..	"	—	Toxin-antitoxin.	No lesion.	28/24 (1.166)
2911..	A	—	None.	—	Normal.
2919..	"	—	Antitoxin.	—	"
2921..	"	—	None.	—	"
2944..	B	—	Toxin-antitoxin.	Induration only.	36+/30 (1.2+)
2945..	"	—	" "	No lesion.	Normal.
3011..	A	—	Toxin.	" "	"
3026..	2870	Normal.	Toxin-antitoxin.	" "	34+/30 (1.133)
3052..	A	—	Antitoxin, 3 times.	—	Normal.
3053..	"	—	" 2 times.	—	"
3066..	"	—	Toxin; antitoxin 1 month later.	Ulcer.	"
3074..	"	—	Antitoxin, twice.	—	"
3122..	C	—	Toxin-antitoxin.	No lesion.	"
3127..	2872	35/30 (1.166)	" "	Ulcer.	30/24 (1.25)
3189..	A	—	" "	No lesion.	27/24 (1.125)
3265..	"	—	" "	" "	30/24 (1.25)
3325..	"	—	" "	Ulcer.	26/24 (1.083)
3333..	3026	34+/30 (1.333+)	" "	No lesion.	26/24 (1.083)
3339..	A	—	" "	Trace of lesion.	Normal.
3378..	D	—	" "	No lesion.	"
3394..	E	—	" "	" "	27/24 (1.125)
3420..	2944	36+/30 (1.2+)	" "	" "	26/24 (1.083)
3422..	"	" "	" "	" "	27/24 (1.125)
3434..	D	—	Toxin-antitoxin.	No lesion.	35+/24 (1.458+)
3437..	"	—	Antitoxin, 2 doses, 3-5 cc. each.	—	Normal.

TABLE I. — *Continued.*

Mother, Data Concerning.					
Designation.	Source: the Number is that of Grandmother.	Resistance.	Preliminary Treatment.	Result.	Resistance of Offspring.
3476..	A	32/29 (1.103)	Toxin-antitoxin.	Ulcer.	26/24 (1.083)
3525..	2444	36+/30 (1.2+)	" "	No lesion.	27/24 (1.125)
3529..	3052	Normal.	" "	Ulcer.	32/24 (1.33)
3544..	D	—	" "	Trace of lesion.	32/24 (1.33)
3577..	3074	Normal.	" "	No lesion.	35/25 (1.4)
3592..	E	—	Antitoxin, 1 dose, 3-5 cc.	—	Normal.
3678..	3074	Normal.	Toxin-antitoxin.	No lesion.	36/25 (1.44)
3685..	E	—	" "	Slough, paralysis.	40+/25 (1.6+)
3691..	3127	30/24 (1.25)	" "	Ulcer.	40+/25 (1.6+)
3718..	F	—	Antitoxin, 2 doses, 3-5 cc. each.	—	Normal.
3732..	3476	26/24 (1.083)	Toxin-antitoxin.	Induration.	35/25 (1.4)
3738..	2602	Normal.	" "	Superficial necrosis.	33+/25 (1.32+)
3762..	2919	"	" "	No lesion.	33+/25 (1.32+)
3772..	3074	"	" "	" "	31/25 (1.24)
3777..	2919	"	" "	Slight reaction.	35+/25 (1.52+)
3788..	3378	"	" "	" "	36/25 (1.44)
3801..	3127	30/24 (1.25)	" "	Ulcer.	35+/25 (1.4+)
3839..	3544	32/24 (1.33)	" "	No lesion.	31/25 (1.24)
3860..	2872	35/30 (1.66)	None.	—	Normal.
3878..	2894	Normal.	Toxin-antitoxin.	No lesion.	33+/25 (1.32+)
3887..	F	—	" "	Induration.	35+/25 (1.4+)
3896..	2921	Normal.	Toxin.	Ulcer.	Normal.
3897..	"	"	Toxin-antitoxin.	Slight reaction.	32+/25 (1.25+)
3931..	3434	35+/24 (1.455+)	" "	Ulcer.	35+/25 (1.4+)
3932..	"	"	" "	"	60+/25 (2.5+)
3934..	"	"	None.	—	Normal.
3945..	3544	32/24 (1.33)	"	—	"
3949..	3678	36/25 (1.44)	Toxin-antitoxin.	Ulcer.	36/25 (1.44)
3965..	3053	Normal.	" "	No lesion.	30+/25 (1.2+)

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TABLE I.—*Concluded.*

Mother, Data Concerning.					Resistance of Offspring.
Designation.	Source: the Number is that of Grandmother.	Resistance.	Preliminary Treatment.	Result.	
4014..	3691	40+/25 (1.6+)	Toxin-antitoxin.	Induration.	35/25 (1.4)
4039..	E	—	None.	—	Normal.
4040..	"	—	"	—	"
4041..	"	—	"	—	"
4042..	"	—	"	—	"
4317..	2872	Normal.	Toxin-antitoxin.	Induration, paralysis.	36+ +/25 (1.44+ +)
4341..	3777	38+/25 (1.52+)	None.	—	Normal.
4359..	F	—	Toxin.	Slough and ulcer.	"
4367..	3738	33+/25 (1.32+)	None.	—	"
4396..	3931	30+ +/25 (1.2+ +)	"	—	"
4400..	3887	35+/25 (1.4+)	"	—	"
4412..	3529	32/25 (1.28)	"	—	"
4482..	3762	33+/25 (1.32+)	None.	—	Normal.
4499..	3691	40+/25 (1.6)	"	—	"
4516..	3839	31/25 (1.24)	"	—	"
4475..	3122	Normal.	Toxin.	Large slough and ulcer.	"
4547..	3887	35+/25 (1.4+)	None.	—	"
4540..	"	35+/25 (1.4+)	"	—	"
4616..	3777	36+/25 (1.44+)	Toxin-antitoxin.	Large slough and ulcer.	40+ +/21 (1.9+ +)

A summary of this table made (A) according to the resistances of the offspring, and (B) according to the treatment and deg of reaction induced in the mother pigs, yields the following results :

TABLE II.

A.	
a. Normal offspring obtained from :	21 mothers not treated = 100 per cent. 7 mothers injected with antitoxin only, 1-3 times = 100%. 4 mothers injected with toxin only = 100 per cent. 5 mothers injected with toxin-anti- toxin mixture = 11.6 per cent.
b. Offspring with resistance $\frac{26-28}{24}$ (1.08 to 1.66) obtained from :	11 mothers treated with toxin-anti- toxin mixtures.
c. Offspring with resistance $\frac{29-60+}{24}$ (1.21-2.5+) obtained from :	27 mothers treated with toxin-anti- toxin mixtures.
B.	
a. Mothers treated with toxin-antitoxin mixtures, and without any result- ing lesion.	3 with normal offspring. 9 with offspring, resistance $\frac{26-28}{24}$ 9 with offspring, resistance $\frac{29-33}{24}$
b. Mothers treated same as under (a) but with slight or transient lesion resulting.	1 with normal offspring. 8 with offspring, resistance $\frac{30-38+}{24}$
c. Mothers treated same as under (a) but with necrosis and ulceration of varying severity.	1 with offspring normal. 2 with offspring, resistance $\frac{26}{24}$ 10 with offspring, resistance $\frac{30-60}{24}$

An analysis of Tables I. and II. show that of the three kinds of treatment, — injection of toxin alone, of antitoxin alone, and of toxin and antitoxin mixed, — only the last is capable of inducing an immunity which is transmitted to the offspring.

The offspring of those females which received toxin alone, even though with severe local reaction, and of those which

received repeatedly doses of antitoxin, remained at the normal level of resistance.* Not all mothers responded, for about 11.9 per cent (five out of forty-three) gave birth to young of normal resistance.

An analysis of the results, by grouping the animals according to the reaction produced by the toxin-antitoxin mixtures, demonstrates that there is no simple relation between reaction, or the amount of non-neutralized toxin in the mixture and the degree of immunity of the offspring. Those mothers which manifest no local signs of reaction may, and frequently do, produce young of increased resistance.

THE MALE DOES NOT TRANSMIT ANY IMMUNITY TO HIS OFFSPRING.

A survey of the literature upon this subject shows an almost unanimous agreement among experimenters that the male does not influence the toxin resistance of his offspring. Yet it seemed desirable to carry out a few experiments to retest this point. The writer in his earlier paper had already shown that the resistance of the offspring of a given mother is not influenced by the father. The following breeding experiments (Table III.) clearly show that there is no reason to doubt the general law first formulated by Ehrlich, — that the male does not transmit immunity and is a negligible factor in our experiments. In this table there are five treated males descended from treated mothers, and possessing considerable resistance. The sixth male was from a resistant litter, but remained untreated. The females were: (1) descended from treated mothers or from treated fathers and mothers, and hence resistant at an early age, but none were treated themselves; (2) normal untreated females. In no case did the offspring of the treated, resistant males possess any more than average or normal resistance.

* See Nos. 3896 and 3897 of Table I. These are of the same litter. One was treated with toxin alone, the other with a toxin-antitoxin mixture.

TABLE III.

Father, Data Concerning.				Mother.		Resistance of Offspring.
Designation.	History.	Treatment and Result.	Resistance.	Designation.	History.	
3944..	Descended from a treated mother, No. 3544.	Toxin-antitoxin. Necrosis.	32+/24	4041...	Untreated. Offspring normal with 1 other male.	Normal.
4100..	Descended from a treated mother, No. 3434.	Toxin-antitoxin. Induration and roughening of skin.	31 + /24	4367... 4341... 4396...	Resistant but untreated. " " " "	Normal. " "
4284..	Descended from a treated mother, No. 3762.	Toxin-antitoxin. Ulcer.	33 + /24	3053... 4040...	Normal. "	Normal. "
3933..	Descended from a treated mother, No. 3434.	Untreated.	30 + /24	4400... 4482...	Resistant but untreated. " "	Normal. "
4368..	Descended from a treated mother, No. 3738.	Toxin-antitoxin. Ulcer.	32 + + /24	3934... 3896... 4042...	Resistant but untreated. Normal; offspring with 2 other males. Normal; offspring with 2 other males, normal. Normal; offspring with 2 other males, normal.	Normal. " "
4467..	Descended from resistant and treated mother, No. 3932.	Toxin-antitoxin. Lesion.	42 + + /24	4547... 4549...	Resistant but untreated. Both parents treated. Resistant but untreated. Both parents treated.	Normal. "

GRADUAL LOSS OF TRANSMITTED PASSIVE IMMUNITY IN
THE OFFSPRING.

The transmission of an increased resistance or immunity by the mother to her offspring is also, without exception, regarded by Ehrlich, Wernicke and others as a passive process in the young. The immunizing antibodies are transmitted through the placenta in utero, and perhaps also to some extent in the milk. This passive immunity is, therefore, of limited duration, most observers regarding it as lasting two to three months. The following table (IV.) shows that the immunity is gradually lost with the increasing age of the offspring. The two control inoculations indicate that in accordance with the great increase in the weight of the animals the L+ dose has risen decidedly. Yet in spite of this increase in weight the L+ dose of the resistant pigs gradually fell so that at the end of three months there was no evidence of more than normal resistance.

TABLE IV.

Designation.	Age in Days.	Weight.	L+ Dose for Guinea-pigs 30 Days Old.	Dose Injected. (\equiv 1 Antitoxic Unit Plus Toxin. The Amount of Toxin over and above the L+ Dose is indicated by the Fraction.)	Result.	Mother.
4645..	43	265	35/25 (1.4)	36/25 (1.44)	Dead, 2¼ days.	3577
4631..	96	478	" "	26/21 (1.24)	" 2 "	"
4633..	113	566	" "	23/21 (1.09)	" 5 "	"
4618..	32	254	43+/25 (1.72+)	43/25 (1.72)	Large slough and ulcer.	3691
4626..	44	258	" "	40/25 (1.6)	Dead, 9½ days.	"
4628..	"	256	" "	35/25 (1.4)	Large slough and ulcer.	"
4619..	91	460	" "	20/21 (1.38)	Dead, 2 days.	"
4627..	116	580	" "	23/21 (1.09)	Large slough and ulcer.	"
4623..	89	421	33/21 (1.56)	27/21 (1.29)	Dead, 2 days.	3732
4614..	58	328	40+25 (1.6+)	30/25 (1.4)	Large ulcer.	3777
4617..	103	530	31/25 (1.24)	26/21 (1.24)	Dead, 1½ days.	3839
4651..	32	257	36+/25 (1.44+)	36/25 (1.44)	Large ulcer.	3878
4649..	119	750	" "	23/21 (1.09)	Large slough and ulcer.	"
4650..	"	650	" "	24/21 (1.14)	" " " "	"
4684..	75	495	35+/25 (1.4+)	21/21 (1.00)	Small ulcer.	3887
4865..	58	268	} 33/21 (1.57)	} 21/21 (1.00)	} Large slough and ulcer.	3732
4867..	"	251				
4868..	"	335				
Controls.						
4640..	115	637	21/21 (1.00)	24/21 (1.14)	Induration, roughening of skin, some loss of hair.	2602
4639..	87	625	" "	22/21 (1.05)	Slough and ulcer.	4039

DISAPPEARANCE OF IMMUNITY IN THE SECOND GENERATION.

All observers cited above have found the grandchildren of immunized females of the usual resistance. Only Vaillard refers to one exception observed by him. This cannot be

regarded as significant, and it may rest upon some experimental error. Of those who worked with diphtheria, Wernicke and Anderson cite illustrations to show loss of immunity in the second generation. The following data are all in accord with earlier observers. A portion of them had been collected before the appearance of Anderson's paper.

In Table I. No. 3934 serves to illustrate the loss of immunity of offspring of an untreated though resistant mother. Nos. 3931, 3932 of the same litter were treated with toxin-antitoxin mixtures, and the resistance of their offspring is very high.

Nos. 4341, 4367, 4396, 4400, 4412, 4482, 4499, 4516, 4547, 4549, 4603, and 4779 are all offspring which inherited a considerable degree of resistance. They were not treated, however, and their offspring have a normal susceptibility.

THE PERSISTENCE OF ACTIVE IMMUNITY IN THE IMMUNIZED FEMALE.

Several authors have called attention to this. Vaillard⁶ traced the immunity through four litters of the same female. Bulloch⁷ found traces of hemolysins in an immunized rabbit three hundred and eighty-seven days after inoculation.

In attempting to determine how long a mother will continue to transmit immunity to her offspring, the investigator frequently finds his animals carried off by some disease, and hence the records obtained from any one individual are likely to be closed at any time. The writer has records of a considerable number of guinea-pigs which transmitted immunity to their offspring for over a year.

The following tables show the uniform level of resistance in the offspring of a normal mother (3053), and in one possessing some active immunity (3434). In this latter animal the single toxin-antitoxin mixture originally injected, to which the immunity is due, produced no lesion, —*i.e.*, the mixture was apparently neutral.

TABLE V.
Guinea-pig No. 3053.

Offspring.	Father.	Test.			Result: Died in	L + Dose cc.
		Date.	Dose of Toxin in cc. + 1 Anti- toxic Unit.	Toxin ¹ used.		
3548..	2891	March 3, 1905.	.30	13 ^b	2 $\frac{3}{8}$ days.	.29
{ 3744 ² ..	3423	June 15, "	"	13 ^c	2 $\frac{1}{2}$ "	"
{ 3746..	"	" 29, "	"	"	2 $\frac{3}{4}$ "	"
{ 3843..	"	Aug. 12, "	"	"	2 $\frac{7}{8}$ "	.30
{ 3842..	"	" " "	.31	"	2 $\frac{1}{2}$ "	"
{ 3841..	"	" " "	.32	"	2 "	"
3964..	"	Nov. 24, "	.24	14 ^a	2 $\frac{1}{2}$ "	.24
4146..	"	Feb. 13, 1906.	"	"	3 $\frac{1}{2}$ "	"
4418..	4037	June 23, "	.25	14 ^b	3 $\frac{1}{4}$ "	.25

¹ The small letters refer to successive bottles of the same test toxin.

² The bracketed numbers include animals of the same litter.

TABLE VI.
Guinea-pig No. 3434 (inoculated Dec. 9, 1904).

Offspring.	Age in Days when Tested.	Father.	Test.			Result.	Normal L + Dose cc.
			Date.	Dose of Toxin in cc. + 1 Anti- toxic Unit.	Toxin used.		
{ 3847..	18	3419	Aug. 8, 1905.	.30	13 ^a	No lesion.	.30
{ 3846..	22	"	Aug. 12, "	.35	"	Transient induration.	"
3932..	30	3647	Oct. 26, "	.30	14 ^a	Large slough.	.24
4103..	40	"	Jan. 13, 1906.	.31	"	" ulcer.	"
4289..	36	3305	May 7, "	.32	14 ^b	" slough.	"
4424..	30	"	July 9, "	.33	"	" "	"
4586..	26	4106	Sept. 12, "	.35	14 ^c	" ulcer.	.25
4736..	32	"	Dec. 29, "	.35	15 ^a	Died in 2 $\frac{1}{2}$ days.	.21
4827..	29	"	March 5, 1907.	.33	"	Died in 3 $\frac{1}{4}$ days.	"

A comparison of the columns "dose of," etc., and "L+ dose" of the tables shows how closely each succeeding litter of 3053 responds to the L+ dose and how much the resistance of the successive litters of No. 3434 is above the normal. Only in the two last litters is the actual L+ dose reached. There it is 1.66 and 1.57 times the average or normal dose. Inasmuch as the immunity dates, roughly speaking, from the time of injection of the toxin-antitoxin mixture, No. 3434 has maintained and transmitted to her young a high level of immunity for over two years, and this because of the single injection of an apparently neutralized toxin-antitoxin mixture.

Tables such as that of No. 3434 could be reproduced in large numbers, but I content myself in giving the periods during which the immunity of the females as manifested in their offspring was under observation. The following figures are obtained by counting the time elapsing between the original injection of the mother and the last litter tested:

One animal observed for a period of 30 months.

"	"	"	"	"	"	26	"
"	"	"	"	"	"	23	"
Three	"	"	"	"	"	22	"
One	"	"	"	"	"	21	"
Two	"	"	"	"	"	20	"
One	"	"	"	"	"	18	"
Three	"	"	"	"	"	17	"
Seven	"	"	"	"	"	16	"
One	"	"	"	"	"	15	"
Four	"	"	"	"	"	13	"

GENERAL SUMMARY.

At the outset I wish to call attention to what is really a new, or at least hitherto unused, method for determining the persistence of active immunity in the body of breeding females.

It is, of course, open to us to withdraw blood from time to time and test its antitoxic value just as the presence of hemolysins, agglutinins, and precipitins is determined. Frequent

withdrawals of blood are not, however, without danger to the animal. Inoculation with toxins is out of the question since every inoculation modifies the existing state of immunity. The determination of minute quantities of antitoxin in the blood serum can be carried out according to the method worked out by E. Marx,¹¹ but this method is even more costly as regards the consumption of guinea-pigs than the method which uses the offspring from time to time.* Another use of this method consists in distinguishing passive from active immunity in the female. The former is not transmitted under the conditions of our experiments, the latter is.

The most important outcome of the resistance tests, covering a period of continuous observation of nearly four years, is the fact that there is apparently no immediate relation between the severity of reaction on the part of the body and the degree of active immunity produced. Animals which have passed through a severe disease due to toxin alone and manifested by loss in weight, fever, extensive local necrosis and ulceration transmit no immunity to their offspring. On the other hand, a single injection of a toxin-antitoxin mixture which produces no local lesion so far as this can be seen or felt and no distinct loss in weight, nor any general symptoms, induces an active immunity which persists for several years. The significance of this fact cannot be made clear without much further experimentation to determine the ultimate fate of apparently neutralized toxin-antitoxin mixtures in the body. I mean by a neutralized mixture of toxin-antitoxin one that produces no manifest local or general lesions recognizable during life in so small an animal as the young guinea-pig. I purposely postpone any discussion of the literature concerning the time required for the union of toxin and antitoxin, and of the different interpretations placed upon the phenomenon connected with this reaction by Ehrlich on the one hand and Arrhenius and

* A preliminary test of the serum of a female guinea-pig, No. 389, inoculated with a nearly neutral toxin-antitoxin mixture in September, 1905, gave the following results: Offspring of March, 1907, showed an L+ dose of 29+/21 or 1.38+. The serum of the mother at this time was less than $\frac{1}{2}$ unit per cubic centimeter, probably $\frac{1}{4}$ unit.

Madsen on the other until experiments now under way have been completed. There can be no question, however, of the fact that toxin-antitoxin mixtures differ materially in their effect upon the body from toxins alone. There may possibly be a slow dissociation of the toxin-antitoxin compound, and a utilization of the large store of toxin introduced under cover of a single antitoxic unit.*

The general belief that a neutral toxin-antitoxin mixture is of no significance in producing active immunity, or in transmitting immunity to offspring, is to be inferred from the fact that the quite extensive literature which has grown up around the testing of antitoxin does not refer to it. The article of Wernicke's was seemingly forgotten. As late as 1899, Behring, in Eulenberg and Samuel's *Lehrbuch d. allg. Therapie u. d. therapeut. Methodik*, p. 998, states quite definitely that the offspring of immunized parents do not possess any increased resistance.†

It is impossible to tell from the context just what methods of immunization were used by Behring, whether he applied toxins and antitoxins separately or in wholly or partly neutralized mixtures. In any case his positive statements were apt to mislead those who were at that time employing the

* The L+ dose of our test toxin is about thirty-eight minimum fatal doses.

† This passage reads as follows: "With the lack of proof of acquired histogenic immunity agrees the fact, observed again and again in my countless toxin tests, that guinea-pigs and mice which are descended from immune parents possess the same sensitiveness to toxins as the offspring of non-immune individuals. In Marburg the guinea-pigs and mice which have served in experiments upon isopathic and anti-toxic immunity are placed in special stables after the end of the experiments, when they are used as breeding animals. The offspring of these I allow to grow up. When these are subsequently used for determining toxin values, a difference in the sensitiveness to toxin between them and the offspring of non-immunized guinea-pigs and mice cannot be recognized in any manner. . . .

"Though I have come to the conclusion that, within a space of time controlled by us, the degree of toxin sensitiveness of any animal species is immovable in successive generations, so that the descendants maintain it even when a generation with altered toxin sensitiveness appears, yet I am far from accepting the fixity of toxin sensitiveness for all times. According to statements made by Tizzoni, there must exist in Bologna a race of rabbits which is far more sensitive to tetanus toxin than those under observation in Germany. I myself have observed races of pigeons varying greatly in sensitiveness to tetanus toxin. In England guinea-pigs appear to have an inherited and transmissible diphtheria immunity of not inconsiderable degree. From a private communication from Ehrlich I know that he has obtained diphtheria immune guinea-pigs from a particular dealer."

antitoxic unit of Ehrlich in the testing of diphtheria antitoxin. He also was evidently not aware of Wernicke's work published four years before, in which the transmission of diphtheria immunity from mother to offspring was clearly demonstrated.

One of the questions raised by the study of transmitted passive immunity relates to the teleological value of such immunity in the protection and strengthening of the young against infectious diseases soon after birth. This is a general biological problem and deserves special discussion in relation to the invasive bacteria and protozoa.

The problem of the part played by the male parent in the transmission of immunity is likewise opened for further investigation. Though he does not transmit directly any passive immunity, yet there is no evidence to show that he does not equally, with the mother, transmit the capacity for producing antibodies, which capacity, according to the figures given in Table I., varies much from family to family, quite independently of the treatment. It is hoped that experiments now under way may shed some light on this problem.

Another problem of considerable practical importance suggested by the foregoing data is the use of similar neutral mixtures for producing an active immunity in the human subject. The chief difficulty to be encountered here would be a toxon-paralysis following the use of mixtures not entirely saturated. Experiments are now under way to determine by methods described in this paper at what antitoxin concentration in the toxin-antitoxin mixture the active immunity of the injected animal may fail to appear, and, therefore, fail to be transmitted to the offspring. If it should appear that a toxin-antitoxin mixture in which the antitoxin is present in much larger amount than is necessary to avoid any local reaction should still lead to an active immunity, the danger of toxon paralysis would be avoided. It would be of great value to substitute for a passive immunity in exposed children an active immunity extending over a considerable period, provided such immunity is attainable as easily and without any more difficulties than in the guinea-pig.

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THE Journal of Medical Research.

(NEW SERIES, VOLUME XI.)

VOL. XVI.

JULY, 1907.

No. 3.

FURTHER STUDIES UPON HYPERSUSCEPTIBILITY AND IMMUNITY.*

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We have shown¹ that horse serum is apparently a bland and harmless substance when injected into a normal guinea-pig, but this injection renders the guinea-pig susceptible to a subsequent injection of horse serum. Ten days must elapse between the first and the second injection for this hypersusceptibility to manifest itself.

The present paper gives the results of our further work upon this interesting phenomenon. We have endeavored to obtain a deeper insight into the cause and nature of hypersusceptibility and have attempted to localize the phenomenon in certain fluids, cells, or organs of the body.

We foresaw last year that the problem of hypersusceptibility has an important bearing upon the question of immunity and expressed the opinion² that "resistance to disease may be largely gained through a process of hypersusceptibility. Whether this increased susceptibility is an

*Read before the American Association of Pathologists and Bacteriologists, Washington, May 9, 1907. Received for publication May 9, 1907.

NOTE. — For details of the experiments in this paper, see Bulletin No. 36, Hygienic Laboratory, U.S. Public Health and Marine Hospital Service.

essential element or only one stage in the process of resistance to disease, must now engage our attention." We cannot escape the conviction that this phenomenon of hypersusceptibility has an important bearing on the prevention and cure of certain infectious processes. Our work this year upon the hypersusceptibility produced by the bacterial proteids strengthens this belief, for our results prove that the phenomenon of hypersusceptibility to certain proteid substances extracted from the bacterial cell is followed by a definite immunity against infection by the same microörganism.

Since our studies last year, several papers have been published which, in the main, have corroborated our findings.

McClintock and King³ gave ten guinea-pigs from 1/250 to one cubic centimeter of horse serum by the stomach and thirteen days later six cubic centimeters of serum either subcutaneously or intraperitoneally without causing symptoms in any of them. They conclude that the sensitizing action of horse serum given by mouth is not nearly so great as when given subcutaneously or intraperitoneally. This is in confirmation of our reported experiments.

Currie⁴ has studied the effect of repeated injections of horse serum in persons admitted for treatment in the city of Glasgow Fever and Smallpox Hospital at Belvidere. He concludes that it is apparent from the facts detailed by him that repeated injections of horse serum induce symptoms of supersensitisation in man; but it is also apparent that the same facts lend no countenance to the suggestion that the death of persons suffering from diphtheria is to be apprehended as the result of repeated injections of anti-diphtheric serum.

Besredka and Steinhardt⁵ studied with much care certain features of hypersusceptibility to horse serum in guinea-pigs; they note that the French serums are much less toxic than those used by Otto in Frankfurt and the serums used by us. Besredka and Steinhardt had a mortality of about twenty-five per cent when five cubic centimeters of serum were given

intraperitoneally at the second injection, whereas death was the rule in our experiments under similar conditions. Most of their work was done with doses of .05 to .25 cubic centimeter given directly into the brain, which either killed or caused grave symptoms in susceptible guinea-pigs. Besredka and Steinhardt lay stress upon the production of "anti-anaphylaxis," which we termed "immunity." They found that a single injection of serum given into the peritoneum of a sensitized guinea-pig conferred immunity to a subsequent injection of .25 cubic centimeter into the brain; in one case the anti-anaphylaxis was present one and a half hours after the injection into the abdominal cavity. They were unable to demonstrate any protective properties in various organs of immune guinea-pigs, confirming our work along the same lines.

Nicolle⁶ found that guinea-pigs were not susceptible to the necrotic action induced by repeated injections of horse serum, as is the case in rabbits; this corresponds with our observations. He also found that daily injections or "spaced" injections, after the method of Arthus, did not induce a high degree of hypersusceptibility in guinea-pigs.

Besredka⁷ questioned whether we should not consider this toxic property of horse serum, as well as its antitoxic power. He suggests that a serum, .05 cubic centimeter of which when given into the brain will kill or cause grave symptoms in a sensitive guinea-pig, should be considered as above the average toxicity and ought to be excluded from use in man.

The work of Otto⁸ on the "Theobald Smith phenomenon," and of von Pirquet and Schick⁹ upon "the serum disease," has been previously referred to.

The sensitizing substance. — We ventured the suggestion in our former publication that the substance that sensitizes the guinea-pig is the same as that which later poisons it; profound changes, perhaps in the central nerve cells, are probably produced by the first injection. Our subsequent work has produced nothing to alter this working hypothesis.

Vaughan¹⁰ advances the theory that the first injection of the strange proteid is broken up into components, one of which is toxic, but that the animal is not poisoned because this breaking up takes place slowly. The cells, however, learn from this lesson how to break up the complex molecule, so that when more of the strange proteid is introduced at the second injection, it is violently rent asunder, quickly liberating large quantities of the toxic principle of the complex molecule.

Vaughan and Wheeler¹¹ have elaborated this explanation by further studies upon egg-white and bacterial proteids split into poisonous and non-poisonous portions. These authors believe that when egg-white, or the non-poisonous portion of egg-white, is injected into a fresh animal certain cells of the body are so influenced that they elaborate a new ferment which, in the form of zymogen, remains in the cell until activated by the second injection, when it is set free and splits up the egg-white in a manner similar to that used by Vaughan in the laboratory. Vaughan and Wheeler believe that the effect induced in the animal is the same as that caused by the poisonous portions of egg-white as they have split it up in the retort.

Currie¹² suggests that the first injection of serum results after an interval in the formation of an antibody. When the second injection of serum is given, after at least ten days from the first, the antibody-producing substance of the second injection of serum and the antibody produced by the first injection come in contact without delay. Their union is rapid; the whole charge of the poisonous substance is quickly set free and the toxic symptoms are sudden and severe.

Besredka and Steinhardt¹³ had, as a working hypothesis, the following: The sensitized guinea-pig which appears in good health has, in spite of its apparent well-being, perhaps, a latent lesion of the brain. A second injection of serum, made into the peritoneal cavity twelve days later, is able to awaken this nervous lesion, resulting in grave symptoms or even death.

In view of these theoretical considerations, it was important to make further studies upon the sensitizing substance in horse serum and other proteid substances.

We were able to demonstrate that the filtrate from horse serum, after precipitation with ammonium sulphate, still possesses sensitizing powers in spite of the fact that this filtrate contains but little of the serum globulin and is very weak in antitoxic value.

Formaldehyd does not appear to modify the sensitizing property in horse serum, though it is capable of destroying the toxic properties of tetanus and diphtheria toxines.

From a limited number of experiments it seems that the sensitizing principle is not dialyzable through a collodion sack when placed in the peritoneal cavity of a guinea-pig, for example:

G.P. No. Cx. Collodion sack containing about 3 cubic centimeters normal horse serum placed in peritoneal cavity.

28 days later, 5 cubic centimeters normal horse serum injected subcutaneously. No symptoms.

1 day later, sack removed.

23 days after removal of sack, 5 cubic centimeters normal horse serum injected subcutaneously. Mild symptoms.

Guinea-pigs may be sensitized by injecting the serum directly into the heart. From this it would appear that the cells lining the peritoneal cavity or the connective tissue cells of the subcutaneous tissue do not, apparently, play a part in the phenomenon of hypersusceptibility.

The toxic principle.—We added a number of different ferments, alkaloids, and simpler chemical substances to horse serum in order to modify, destroy, or neutralize its toxic action. All these attempts proved unavailing. The following ferments were used: Taka-diastrase, pancreatin, rennin, myrosin, invertin, emulsin, pepsin in acid solution, pepsin in alkaline solution, ingluvin, malt, and papain. The ferments were added to the horse serum and allowed to stand at 15° C. over night.

The following alkaloids were added to serum in amounts less than the toxic dose for the animal, but apparently without modifying the symptoms in any way: Atropin, strychnin, morphin, and caffen.

Calcium chlorid, sodium nitrate, sodium chlorid, magnesium sulphate, and ammonium sulphate were without any modifying action upon the toxic property.

We thought perhaps by mixing the serum with ox-bile, or by shaking it up with animal charcoal or yeast cells, that the toxic principle might be altered in some way; these substances were found to be without effect.

In view of the fact that formaldehyd has a destructive action upon such "haptin" substances as tetanus and diphtheria toxines, and in further view of the fact that the sensitizing and toxic principles of horse serum seem to belong to the haptine group of substances in the sense used by Ehrlich, it became interesting to determine what effect formaldehyd would have upon hypersusceptibility produced by horse serum.

The formaldehyd was added to normal horse serum in various proportions and allowed to stand for different lengths of time before injecting into guinea-pigs without, however, modifying the symptoms to any appreciable extent.

Calcium chlorid. — Netter¹⁴ has shown that when one gram of calcium chlorid is given on the day of injection, and on the two following days, the number of children showing eruption following the injection of serum is greatly reduced. We thought perhaps this salt might have some influence upon the phenomenon produced in guinea-pigs by two injections of horse serum. We fed calcium chlorid in small amounts to a number of guinea-pigs for various lengths of time without, however, modifying either the sensitizing or toxic principle in the serum. However calcium chlorid may modify the occurrence of rashes in children following a single injection of serum, it does not influence to any marked extent the toxic effect in guinea-pigs of a second injection of serum given fourteen days after the first injection.

The toxic principle is not affected by freezing at 15° F.

Is the toxic principle specific? — The toxic action is quantitatively specific so far as various blood serums are concerned. That is, a guinea-pig sensitized with horse serum is more susceptible to a subsequent injection of horse serum than to a subsequent injection of the blood serum of cattle, sheep, cats, dogs, hogs, etc. The specific character of the hypersusceptibility is more apparent when proteid substances of quite different origin are used at the first and second injections. For example, guinea-pigs sensitized with horse serum do not react at all to subsequent injections of peptone, vegetable proteid extracts, egg albumen, or milk.

The converse of the above is also true, that guinea-pigs sensitized with subcutaneous injections of these various proteid substances do not react to a subsequent injection of horse serum.

Other albuminous substances. — As soon as we concluded that it is probably the proteid substance in horse serum that is chiefly concerned in sensitizing and poisoning the guinea-pigs, we thought of other proteid substances obtained from widely different sources.

We have found that hemoglobin, egg albumen, milk, and extract of peas are quite as active as horse serum. Peptone seems to have slight sensitizing and poisonous properties; leucin and tyrosin none at all. The reaction following the second injections of proteid matter in the guinea-pig appears then to be common to all the higher forms of albuminous substances, no matter from what source. It occurs to us that this phenomenon of hypersusceptibility in the guinea-pig may be used as a physiological test to distinguish true proteid substances from the lower forms of nitrogenous compounds. It would be interesting to determine whether the synthetic peptids and polypeptids of Fisher sufficiently approach the true proteid molecular structure to induce hypersusceptibility in the guinea-pig.

HEMOGLOBIN *versus* HEMOGLOBIN.

No. Exp.	First Injection.	Interval in Days.	Second Injection.	Result.
1	1 cc. hemoglobin washed 20 times, subcutaneously.	22	6 cc. hemoglobin, intraperitoneally.	Marked symptoms.
2	5 cc. hemoglobin washed 20 times, subcutaneously.	22	6 cc. hemoglobin, intraperitoneally.	Severe symptoms.
3	1 cc. hemoglobin washed 20 times, subcutaneously.	22	6 cc. hemoglobin, intraperitoneally.	Dead, 5 min.
4	3 cc. hemoglobin washed 20 times, subcutaneously.	22	6 cc. hemoglobin, intraperitoneally.	Slight symptoms.
5	5 cc. hemoglobin washed 20 times, subcutaneously.	22	6 cc. hemoglobin, intraperitoneally.	Very severe symptoms.

The hemoglobin was obtained by dissolving the washed red corpuscles of a normal horse in distilled water. The red corpuscles for the hemoglobin solution used at the first injection, in order to sensitize the guinea-pigs, were washed and centrifugalized twenty times in order to surely wash away all traces of serum, the smallest remaining quantities of which might have confused the results. The hemoglobin used at the second injection was dissolved from red corpuscles washed four times.

EGG ALBUMEN *versus* EGG ALBUMEN.

No. G.P.	First Injection.	Interval in Days.	Second Injection.	Result.
4061 cc. egg albumen subcutaneously.	22	6 cc. saturated solu- tion egg albumen in salt solution, intraperitoneally.	Dead, 30 min.
4075 cc. egg albumen subcutaneously.	22	6 cc. saturated solu- tion egg albumen in salt solution, intraperitoneally.	Dead, 18 min.
408	1 cc. egg albumen subcutaneously.	22	6 cc. saturated solu- tion egg albumen in salt solution, intraperitoneally.	Dead, 25 min.
409	3 cc. egg albumen subcutaneously.	22	6 cc. saturated solu- tion egg albumen in salt solution, intraperitoneally.	Dead, 25 min.
410	5 cc. egg albumen subcutaneously.	22	6 cc. saturated solu- tion egg albumen in salt solution, intraperitoneally.	Dead, 20 min.
732	1 cc. egg albumen + salt solution sub- cutaneously.	21	6 cc. egg albumen + salt solution equal quantities, intraperitoneally.	Severe symptoms.

MILK *versus* MILK.

No. G.P.	First Injection.	Interval in Days.	Second Injection.	Result.
401	3 cc. milk, filtered through porcelain, subcutaneously.	26	10 cc. bottom milk intraperitoneally.	Slight symptoms.
402	1 cc. milk, filtered through porcelain, subcutaneously.	26	10 cc. bottom milk intraperitoneally.	Slight symptoms.
4035 cc. milk, filtered through porcelain, subcutaneously.	26	10 cc. bottom mi.k intraperitoneally.	Slight symptoms.
4041 cc. milk, filtered through porcelain, subcutaneously.	26	10 cc. bottom milk intraperitoneally.	Slight symptoms.
7065 cc. fresh whole milk subcutaneously.	31	10 cc. fresh whole milk intraperito- neally.	Dead, 20 min.
707	1 cc. fresh whole milk subcutaneously.	31	10 cc. fresh whole milk intraperito- neally.	Very severe symp- toms.
708	3 cc. fresh whole milk subcutaneously.	31	10 cc. fresh whole milk intraperito- neally.	Very severe symp- toms.
709	5 cc. fresh whole milk subcutaneously.	31	10 cc. fresh whole milk intraperito- neally.	Very severe symp- toms.
7371 cc. fresh whole milk subcutaneously.	31	10 cc. fresh whole milk intraperito- neally.	Very severe symp- toms.
Control ...	6 cc. fresh whole milk (acid), intraperitoneally.			No symptoms.

PEAS *versus* PEAS.

No. G.P.	First Injection.	Interval in Days.	Second Injection.	Result.
4161 cc. watery extract of peas, 24 hrs. at 15° C. (acid) filtered through porcelain, subcutaneously.	26	10 cc. watery extract of peas, 3 days at 15° C., filtered through porcelain, intraperitoneally.	Marked symptoms.
4175 cc. watery extract of peas, 24 hrs. at 15° C. (acid) filtered through porcelain, subcutaneously.	26	10 cc. watery extract of peas, 3 days at 15° C., filtered through porcelain, intraperitoneally.	Dead, 7 hrs. 30 min.
418	1 cc. watery extract of peas, 24 hrs. at 15° C. (acid) filtered through porcelain, subcutaneously.	26	10 cc. watery extract of peas, 3 days at 15° C., filtered through porcelain, intraperitoneally.	Marked symptoms.
419	3 cc. watery extract of peas, 24 hrs. at 15° C. (acid) filtered through porcelain, subcutaneously.	26	10 cc. watery extract of peas, 3 days at 15° C., filtered through porcelain, intraperitoneally.	Dead, 4 hrs.
420	5 cc. watery extract of peas, 24 hrs. at 15° C. (acid) filtered through porcelain, subcutaneously.	26	10 cc. watery extract of peas, 3 days at 15° C., filtered through porcelain, intraperitoneally.	Dead, 2 hrs.
Control ...	10 cc. watery extract of peas, 3 days at 15° C., filtered through porcelain, intraperitoneally.			No symptoms.
Control ...	10 cc. watery extract of peas, 3 days at 15° C., filtered through porcelain, intraperitoneally.			No symptoms.

PEPTONE *versus* PEPTONE.

No. G.P.	First Injection.	Interval in Days.	Second Injection.	Result.
492	5 cc. half saturated solution peptone, subcutaneously.	21	6 cc. saturated solution peptone, intraperitoneally.	Slight symptoms.
490	1 cc. half saturated solution peptone, subcutaneously.	21	6 cc. saturated solution peptone, intraperitoneally.	Mild symptoms.
491	3 cc. half saturated solution peptone, subcutaneously.	21	6 cc. saturated solution peptone, intraperitoneally.	Marked symptoms.
458	1 cc. half saturated solution peptone, subcutaneously.	21	6 cc. saturated solution peptone, intraperitoneally.	Marked symptoms.
748	1 cc. half saturated solution peptone, subcutaneously.	21	6 cc. half saturated solution peptone, intraperitoneally.	Marked symptoms.
749	5 cc. half saturated solution peptone, subcutaneously.	21	6 cc. half saturated solution peptone, subcutaneously.	No symptoms.
750	1 cc. half saturated solution peptone, subcutaneously.	21	6 cc. half saturated solution peptone, subcutaneously.	Marked symptoms.
751	3 cc. half saturated solution peptone, subcutaneously.	21	6 cc. half saturated solution peptone, intraperitoneally.	Marked symptoms.
752	5 cc. half saturated solution peptone, subcutaneously.	21	6 cc. half saturated solution peptone, subcutaneously.	Marked symptoms.
Control ...	6 cc. half saturated solution peptone, heated, subcutaneously.			No symptoms.

TYROSIN *versus* TYROSIN.

No. G.P.	First Injection.	Interval in Days.	Second Injection.	Result.
739002 gram watery solution tyrosin, subcutaneously.	21	6 cc. tyrosin (.1 gram + 50 cc.) watery solution, intraperitoneally.	No symptoms.
74001 gram watery solution tyrosin, subcutaneously.	21	20 cc. tyrosin (.1 gram + 50 cc.) watery solution, intraperitoneally.	No symptoms.
74102 gram watery solution tyrosin, subcutaneously.	21	10 cc. tyrosin (.1 gram + 50 cc.) watery solution, intraperitoneally.	No symptoms.
Control....	6 cc. tyrosin (.1 gram + 50 cc.) watery solution, intraperitoneally, into a fresh guinea-pig.			No symptoms.

LEUCIN *versus* LEUCIN.

No. G.P.	First Injection.	Interval in Days.	Second Injection.	Result.
74502 gram leucin ² in watery solution, subcutaneously.	17	20 cc. (.2 gram + 50 cc.) watery solution, intraperitoneally.	No symptoms.
74602 gram leucin in watery solution, subcutaneously.	17	6 cc. (.2 gram + 50 cc.) watery solution, intraperitoneally.	No symptoms.
74702 gram leucin in watery solution, subcutaneously.	17	10 cc. (.2 gram + 50 cc.) watery solution, intraperitoneally.	No symptoms.
Control ...	6 cc. leucin (.2 gram + 50 cc.) watery solution, intraperitoneally, into a fresh guinea-pig.			No symptoms.

Other blood serums and other albuminous substances are also toxic. — So much of our work has been done with horse serum that we desire to record some further experiments with the blood serums of other animals. We confirm and extend our previous work that the same reactions may be induced in the guinea-pig with the blood serums of various animals, such as the dog, ox, sheep, cat, and hog.

No. G.P.	First Injection.	Interval in Days.	Second Injection.	Result.
	Subcutaneously, $\frac{1}{10}$ cc. Serum of		Intraperitoneally 6 cc. Serum of	
461.....	Ox.	37	Ox.	Dead, 120 min.
462.....	"	37	"	Marked symptoms.
463.....	"	37	"	Severe symptoms.
465.....	"	37	"	Severe symptoms.
466.....	Sheep.	37	Sheep.	Slight symptoms.
467.....	"	37	"	Dead, 110 min.
468.....	"	37	"	Severe symptoms.
469.....	"	37	"	Severe symptoms.
470.....	"	37	"	Dead, 12 hrs.
471.....	Hog.	37	Hog.	Mild symptoms.
472.....	"	37	"	Dead, 12 hrs.
473.....	"	37	"	Dead, 1 hr.
474.....	"	37	"	Severe symptoms.
475.....	"	37	"	Severe symptoms.
476.....	Dog.	70	Dog.	Dead, 60 min.
477.....	"	70	"	Dead, 120 min.
478.....	"	70	"	Dead, 20 min.
479.....	"	70	"	Dead, 65 min.
480.....	"	70	"	Dead, 70 min.
481.....	Cat.	70	Cat.	Dead, 120 min.
482.....	"	70	"	Dead, 50 min.
483.....	"	70	"	Dead, 120 min.
484.....	"	70	"	Dead, 50 min.
485.....	"	70	"	Dead, 65 min.

Comparative toxicity of untreated and refined antitoxic serum. — It has long been known that diphtheria antitoxine is precipitated from the serum with the globulins and many attempts have been made to separate the antitoxine from the non-antitoxic substances contained in the serum.

Gibson¹⁵ has evolved a practical method of concentrating and refining diphtheria antitoxic serum. Part of the process consists in placing the one-half saturation of ammonium sulphate precipitate derived from the antitoxic serum in saturated sodium chlorid solution. This dissolves a portion of the globulins with all the antitoxine. In this way the nucleo-proteids and insoluble globulins present in the first precipitate are eliminated. The soluble globulins are precipitated by acetic acid, filtered, partially dried, and finally placed in a sack of parchment membrane and dialyzed in running water. This antitoxic solution of soluble globulins is then rendered neutral and sufficient sodium chlorid added to make it isotonic.

Park and Throne¹⁶ find, from a comparative study of one hundred cases, that the removal of a considerable portion of the non-antitoxic globulins from the serum by the Gibson method eliminates much of the deleterious matter from the serum, so that severe rashes, joint complications, fever, and other constitutional disturbances are less likely to occur from the antitoxic globulins than from the antitoxic serum from which they were obtained.

We asked ourselves the question whether the precipitated and refined serum is less toxic to sensitized guinea-pigs than the untreated serum from which it was made. Park kindly furnished us some of the precipitated serum and the corresponding untreated serum from which it was made in order to carry out these tests.

By using the precipitated serum and the untreated serum from the same horse we were able to show by a number of comparative experiments that refined antitoxic serum precipitated and dialyzed in accordance with the Gibson method is quite as toxic, bulk for bulk, as the untreated serum from which it has been obtained. We must, however,

consider that the treatment to which the serum is subjected in accordance with the Gibson method concentrates its antitoxic power about twice. There is, therefore, a distinct advantage gained, so far as bulk is concerned, in giving a corresponding number of antitoxic units, for the serum reaction in children depends partly upon the quantity of serum given.

Comparative toxicity of different horse serums. — Besredka and Steinhardt¹⁷ believe that the French horse serums are much less toxic than those used by Otto¹⁸ and the serums used by us. Besredka and Steinhardt had a mortality of about twenty-five per cent when five cubic centimeters of serum were injected intraperitoneally at the second injection, whereas Otto's and our percentage under similar conditions was much higher. Besredka kindly sent us a quantity of "serum antidiphtérique" prepared at the Pasteur Institute and this serum was injected into a series of guinea-pigs in order to compare its toxicity with the normal horse serum of our own horse that we have used so much in these experiments.

TOXICITY OF "SERUM ANTIDIPHTÉRIQUE" (PASTEUR INSTITUTE).

No. G.P.	First Injection.	Interval in Days.	Second Injection.	Result.
7768..	.142 cc. toxine 5 + $\frac{1}{10}$ cc. antitoxic horse serum (S. spl. .1351), subcutaneously.	76	5 cc. antitoxic horse serum (Pasteur Institute), intraperitoneally.	Dead, 11 min.
7774..	.142 cc. toxine 5 + $\frac{1}{10}$ cc. antitoxic horse serum (S. spl. .1351), subcutaneously.	76	5 cc. antitoxic horse serum (Pasteur Institute), intraperitoneally.	Dead, 10 min.
7773..	.142 cc. toxine 5 + $\frac{1}{10}$ cc. antitoxic horse serum (PD. .08022), subcutaneously.	83	5 cc. antitoxic horse serum (Pasteur Institute), intraperitoneally.	Dead, 15 min.
7776..	.142 cc. toxine 5 + $\frac{1}{10}$ cc. antitoxic horse serum (PD. .08022), subcutaneously.	83	5 cc. antitoxic horse serum (Pasteur Institute), intraperitoneally.	Dead, 9 min.
7849..	.142 cc. toxine 5 + $\frac{1}{10}$ cc. antitoxic horse serum (A. 192), subcutaneously.	58	5 cc. antitoxic horse serum (Pasteur Institute), intraperitoneally.	Dead, 19 min.
440..	.0006 gram tetanus toxine A + $\frac{1}{10}$ cc. antitoxic horse serum (Hæchst), subcutaneously.	56	5 cc. antitoxic horse serum (Pasteur Institute), intraperitoneally.	Dead, 10 min.
451..	.0006 gram tetanus toxine A + $\frac{1}{10}$ cc. antitoxic horse serum (Park), subcutaneously.	56	5 cc. antitoxic horse serum (Pasteur Institute), intraperitoneally.	Dead, 10 min.

TOXICITY OF NORMAL HORSE SERUM (OUR ROAN).

No. G.P.	First Injection.	Interval in Days.	Second Injection.	Result.
7845..	.24 cc. toxine 9 + $\frac{1}{10}$ cc. antitoxic horse serum (A. 192), subcutaneously.	58	5 cc. normal horse (roan) serum, intraperitoneally.	Dead, 20 min.
7850..	.24 cc. toxine 9 + $\frac{1}{10}$ cc. antitoxic horse serum (A. 142), subcutaneously.	58	5 cc. normal horse (roan) serum, intraperitoneally.	Dead, 18 min.
7775..	.24 cc. toxine 9 + $\frac{1}{10}$ cc. antitoxic horse serum (PD. .08022), subcutaneously.	80	5 cc. normal horse (roan) serum, intraperitoneally.	Dead, 35 min.
444..	.0006 gram tetanus toxine A + $\frac{1}{10}$ cc. antitoxic horse serum (Hæchst), subcutaneously.	56	5 cc. normal horse (roan) serum, intraperitoneally.	Very severe symptoms.
447..	.0006 gram tetanus toxine A + $\frac{1}{10}$ cc. antitoxic horse serum (M. 2122), subcutaneously.	56	5 cc. normal horse (roan) serum, intraperitoneally.	Very severe symptoms.

NOTE. — Where "toxine" is mentioned in the tables, diphtheria toxine is meant, unless otherwise stated.

It is perfectly evident from the above that our results upon the comparative toxicity of the French and American serums do not agree with those reported by Besredka and Steinhardt. With us the French serums are perhaps somewhat more toxic than our own. We believe these contradictory results are due to other causes than the relative toxicity of the different serums. It is not likely that these differences are due to varying susceptibility of the different breeds of guinea-pigs. We have found little difference between guinea-pigs obtained from five or six different sources. Further, we have sometimes been struck with the fact that guinea-pigs from our own stock and raised under precisely similar conditions show striking differences of degree in the reaction to the second injection. For instance, all the guinea-pigs sensitized with toxine-antitoxine mixtures upon a certain date will subsequently prove exceedingly sensitive and most of them will die at the second injection, whereas another lot of guinea-pigs similarly sensitized at another time will prove much less susceptible at the second injection. So far as we are able to judge, this difference of toxicity depends upon something connected with the sensitizing action and not with the variety of horse serum given at the second injection.

The immunity to hypersusceptibility or "anti-anaphylaxis."—The immunity produced against the toxic action by repeated injections of horse serum has been called anti-anaphylaxis by Besredka and Steinhardt.¹⁹ From our subsequent work we learn that this immunity is relatively not quite as lasting and definite as many instances of active immunity seen in the laboratory against bacterial infections. Guinea-pigs that have received a number of prior injections of horse serum may again show symptoms when re-injected with large amounts. The symptoms in such cases are usually mild and death has never occurred in an "immunized" guinea-pig as a result of subsequent injections with horse serum.

Examples.

G.P. No. 410:

Jan. 15, 1906. 1 cubic centimeter antitoxic horse serum (Natl. VIII., 17), subcutaneously. No symptoms.

- Jan. 23, 1906. 1 cubic centimeter antitoxic horse serum (Natl. VIII., 17), subcutaneously. No symptoms.
- Feb. 8, 1906. 6 cubic centimeters antitoxic horse serum (Natl. VIII., 17), subcutaneously. No symptoms.
- Feb. 14, 1906. 6 cubic centimeters antitoxic horse serum (Natl. VIII., 17), subcutaneously. No symptoms.
- Feb. 23, 1906. 6 cubic centimeters antitoxic horse serum (Natl. VIII., 17), subcutaneously. No symptoms.
- Mar. 29, 1906. 6 cubic centimeters antitoxic horse serum (Natl. VIII., 18), subcutaneously. No symptoms.
- April 18, 1906. 6 cubic centimeters antitoxic horse serum (Natl. VIII., 17), subcutaneously. No symptoms.
- May 16, 1906. 6 cubic centimeters normal horse (roan) serum. No symptoms.
- May 18 to June 27, 1906. 1 cubic centimeter daily normal horse (roan) serum. No symptoms.
- Sept. 7, 1906. 6 cubic centimeters normal horse (roan) serum, intraperitoneally. No symptoms.

G.P. No. 430:

- May 18 to June 27, 1906. 1 cubic centimeter normal horse (roan) serum, subcutaneously, daily. No symptoms.
- Feb. 27, 1907. 6 cubic centimeters normal horse (roan) serum, intraperitoneally. Severe symptoms.

G.P. No. 427:

- May 18 to June 26, 1906. 1 cubic centimeter normal horse (roan) serum, daily, subcutaneously. No symptoms.
- Feb. 27, 1907. 6 cubic centimeters normal horse (roan) serum, intraperitoneally. Severe symptoms.

G.P. No. 4426.

- Jan. 10, 1906. .002 cubic centimeter toxine No. 5.
- Jan. 18, 1906. 6 cubic centimeters normal horse (No. 15) serum, intraperitoneally. No symptoms.
- Feb. 14, 1906. 6 cubic centimeters antitoxic horse serum (Natl. VIII., 17), intraperitoneally. Symptoms (?)
- Feb. 23, 1906. 6 cubic centimeters antitoxic horse serum (Natl. VIII., 17), intraperitoneally. No symptoms.
- Mar. 29, 1906. 6 cubic centimeters antitoxic horse serum (Natl. VIII., 17), intraperitoneally. Mild symptoms.
- April 18, 1906. 6 cubic centimeters antitoxic horse serum (Natl. VIII., 18), intraperitoneally. No symptoms.
- May 16, 1906. 6 cubic centimeters normal horse (roan) serum, intraperitoneally. Severe symptoms.
- May 18 to June 27, 1906. 1 cubic centimeter normal horse (roan) serum, intraperitoneally, daily, except Sunday; 22 injections. No symptoms.

Jan. 25, 1907. 6 cubic centimeters normal horse (15) serum. No symptoms.

Mar. 26, 1907. 6 cubic centimeters normal horse (roan) serum. No symptoms.

(For other instances of this variation in resistance, see Part IX., pages 59 to 62, Bulletin 29, Hygienic Laboratory, U.S. Public Health and Marine Hospital Service.)

Maternal transmission of hypersusceptibility and immunity. — In our previous work we showed that hypersusceptibility to the toxic effects of horse serum may be transmitted from the mother guinea-pig to her young. Later, one of us (Anderson) showed that the female guinea-pig may transmit hypersusceptibility to horse serum and immunity to diphtheria toxine at the same time. On account of certain analogies between the reaction to tuberculin and the toxic action of horse serum, we have made further studies along these lines. In this paper we shall refer only to our studies upon the transmission of hypersusceptibility and immunity to the toxic action of horse serum, leaving related studies with tuberculosis and tuberculine for a future publication.

Our present studies corroborate the fact that hypersusceptibility to the toxic action of horse serum always is transmitted from the mother guinea-pig to her young. This function is solely maternal; the male takes no part whatever in the transmission of these acquired properties. Whether this maternal transmission is hereditary or congenital cannot be definitely stated.

We are able to exclude the milk as a factor in transmitting the hypersusceptibility to the toxic action of horse serum by a series of exchange experiments. "Exchange" experiments consist in at once placing guinea-pigs born of a susceptible mother to nurse with an untreated female and, in exchange, the young of the untreated female are at the same time placed to nurse with the susceptible female. From these "exchange" experiments we learn that the hypersusceptibility is not transferred to the young in the milk.

We also learn from our experiments that hypersusceptibility may be transmitted from mother to young whether the

mother is sensitized before or after conception. The fact that this influence may take place after conception might be taken to indicate that the transmission is congenital and not hereditary.

The relation of hypersusceptibility to various influences. — We have already shown that hypersusceptibility to the action of horse serum in the guinea-pig has no evident relation to hemolysis or precipitins.

Aggressines. — The work of Bail upon aggressines induced us to try whether a similar action may explain hypersusceptibility. Normal horse serum was injected into the peritoneal cavity of a normal guinea-pig; this produced no symptoms. Two hours later the animal was chloroformed and about six cubic centimeters of the fluid taken from the peritoneal cavity and injected into a sensitized guinea-pig; the symptoms were not modified in any way, the pig dying in about twenty minutes.

Normal horse serum was then injected into the peritoneal cavity of a sensitized guinea-pig. The animal developed typical symptoms and died in fifteen minutes as a result of the second injection. About four cubic centimeters of the peritoneal fluid from this animal was injected into the peritoneal cavity of a normal guinea-pig without, however, causing any symptoms.

A sensitized guinea-pig was given six cubic centimeters of normal horse serum, which was followed by characteristic symptoms and death in thirty minutes. Three cubic centimeters of the peritoneal fluid of this pig was collected and injected into a sensitized guinea-pig, causing severe symptoms, but not death.

From the above example it seems that this phenomenon of hypersusceptibility bears no evident relation to the aggressines.

Methemaglobin poisoning. — The symptoms in the guinea-pig somewhat resemble methemaglobin poisoning. We are indebted to Assistant Surgeon A. M. Stimson, of the U.S.

Public Health and Marine Hospital Service, for comparative spectroscopic studies of the blood of normal guinea-pigs and of the blood of susceptible guinea-pigs immediately after death caused by a second injection of horse serum. No methemaglobin was found; only the bands corresponding to oxyhemaglobin were seen in the blood of the guinea-pigs examined immediately after death.

Oxygen has no influence.—A sensitized guinea-pig was inoculated with horse serum and at once placed in an almost pure atmosphere of oxygen. Another sensitized guinea-pig (not reinoculated) was placed under the same bell-jar as a control. The inoculated pig developed symptoms and was dead in thirty-five minutes. The control animal showed no unusual manifestations after thirty minutes in the atmosphere of oxygen; it was then given an injection of horse serum and immediately replaced under the bell-jar. It developed characteristic symptoms and died in fifteen minutes.

The influence of diphtheria toxine upon hypersusceptibility. — The question was raised by both Otto and ourselves as to the influence of the diphtheria toxine in accentuating the phenomenon of hypersusceptibility.

While guinea-pigs may be sensitized with fresh normal horse serum alone, it seemed to us and also to Otto that a greater degree of hypersusceptibility is produced when sensitized with a mixture of diphtheria toxine and antitoxic horse serum than when the horse serum alone is given at the first injection. It seems, however, that this is by no means always the case.

Twenty guinea-pigs were sensitized with 1.250 cubic centimeter antitoxic horse serum. One-half the animals received, in addition, .2 cubic centimeter of diphtheria toxine (MLD. = .006). After thirty-one days all the guinea-pigs were tested with three cubic centimeters normal horse serum into the peritoneal cavity. The addition of the toxine appeared to have no appreciable influence upon the sensitizing power of the serum, in this experiment, as the severity

of the symptoms and the number of deaths was about equal in both series.

Tetanus toxine. — Besredka and Steinhardt²⁰ intimate that guinea-pigs sensitized with a mixture of tetanus toxine and antitetanic serum are not sensitive to subsequent injections of horse serum. These scientists, however, suggest that their failures on this point may have been due to the small amount of horse serum used at the first injection, viz., 1/10,000 and 1/100,000 cubic centimeter.

We tested some of our used tetanus guinea-pigs to determine this point and found that tetanus toxine does not apparently influence the phenomenon of hypersusceptibility to horse serum. The guinea-pigs were sensitized with .0006 gram of a dried tetanus toxine, which represents one hundred minimal lethal doses, plus various amounts of antitetanic serum. All those tested reacted to a second injection of horse serum.

Spleen and thyroid. — While we believe that this reaction is probably localized in the central nervous system, several experiments were undertaken to determine what influence the spleen and the thyroid gland might have upon the hypersusceptibility produced by the injection of horse serum.

The removal of the thyroid gland or the spleen either before or after an inoculation of horse serum does not prevent the phenomenon of hypersusceptibility.

It is of interest to note that the guinea-pigs upon which splenectomy was performed lost much hair and became reduced in weight, although the appetite seemed to remain good.

Feeding experiments with cooked meat. — In our former work we showed that guinea-pigs may be sensitized by feeding them blood serum or meat. We know that heating blood serum to 100° C. for fifteen minutes is sufficient to destroy its toxic action. We then asked ourselves the question, Would the heating of the meat prevent the

sensitizing action? We made a number of experiments indicating that heat does destroy this property of meat, so far as the guinea-pig is concerned. In these experiments well cooked horse meat was used. It was heated in the hot air sterilizer at 110° C. for thirty minutes. Two grams of it were fed to the animals daily from June fifteenth to June thirtieth. Each guinea-pig, therefore, received thirty-two grams of the cooked meat. None of them showed any symptoms when injected eighteen days later with six cubic centimeters of normal horse serum.

Feeding experiments with raw beef.—We know that guinea-pigs fed with horse serum or horse meat are susceptible to subsequent injections of horse serum and we are now able to show by the following series of experiments that guinea-pigs fed with beef are susceptible to subsequent injections of cattle serum.

No. G.P.	First Treatment.	Interval in Days.	Second Treatment.	Result.
301...	Fed 2 grams dried beef daily for 23 days.	19	6 cc. cow serum, intraperitoneally.	Mild symptoms.
302...	Fed 2 grams dried beef daily for 23 days.	19	6 cc. cow serum, intraperitoneally.	Mild symptoms.
303...	Fed 2 grams dried beef daily for 23 days.	19	6 cc. cow serum, intraperitoneally.	Severe symptoms.

Result of cardiac injections.—We have shown that guinea-pigs may readily be sensitized by subcutaneous or intraperitoneal injections, and that the second injection produces symptoms when the serum is injected either under the skin or into the peritoneal cavity.

Besredka and Steinhardt have shown that the injection of serum into the brain of a sensitized guinea-pig is very poisonous. We are able to confirm this observation. We then asked ourselves the question, Can guinea-pigs be sensitized by injecting the horse serum directly into the heart? And can sensitized guinea-pigs be poisoned by such injections directly into the circulation? We are now enabled to

answer these questions affirmatively in view of the following experiments:

No. G.P.	First Injection.	Interval in Days.	Second Injection.	Result.
7077.....	.142 cc. toxine No. 5 + .118 cc. antitoxic horse serum (NY BH 17), subcutane- ously.	131	1.5 cc. normal horse (roan) serum, into heart.	Dead, 3 min.
7692.....	.142 cc. toxine No. 5 + .118 cc. antitoxic horse serum (A 247), subcutane- ously.	35	.01 cc. normal horse (roan) serum, into heart.	Dead, 55 min.
7630.....	.142 cc. toxine No. 5 + .118 cc. antitoxic horse serum (A ppt. 31), subcutaneously.	44	1 cc. normal horse (roan) serum, into heart.	Dead, 3½ min.
7632.....	.142 cc. toxine No. 5 + .118 cc. antitoxic horse serum (A ppt. 31), subcutaneously.	44	1 cc. normal horse (roan) serum, into heart.	Dead, 3 min.
7601.....	.142 cc. toxine No. 5 + .118 cc. antitoxic horse serum (A 247), subcutane- ously.	35	1 cc. normal horse (roan) serum, into heart.	Dead, 3 min.
Control..	2 cc. normal horse (roan) serum, into heart.	No symptoms.
	Same guinea-pig.	20 days later.	6 cc. normal horse (roan) serum, in- traperitoneally.	Marked symptoms.

These experiments indicate that the endothelial cells lining the peritoneal cavity or the connective tissue cells of the subcutaneous tissue do not necessarily play a part in the phenomenon we are studying.

Time. — Guinea-pigs remain susceptible to the toxic action of horse serum a very long time. In one case four hundred and eighty days elapsed between the first and the second injections.

The effect of first injections of horse serum into guinea-pigs. — Theobald Smith²¹ stated that guinea-pigs which have received no preliminary doses of serum may die of a first

injection: of fifty-eight guinea-pigs receiving three to five cubic centimeters of diphtheria antitoxine with no preliminary treatment, nine died and forty-nine showed no effect, making 15.5 per cent of the guinea-pigs reacting to the first injection of horse serum. In reply to a question, Smith stated that he did not know whether or not the animals were the young of guinea-pigs that had been treated.

We have injected many guinea-pigs with horse serum and have never noted symptoms or death to follow the first injection and cannot help but believe that the results obtained by Smith are explained by our studies upon the maternal transmission of hypersusceptibility.

Hypersusceptibility and immunity produced by bacterial proteids. — Experimental studies with the bacterial proteids are of the greatest importance on account of the practical uses to which results along this line may lead. Our conviction that the phenomenon of hypersusceptibility which we have been studying in the guinea-pig has a deep significance in general pathology, especially in the problem of immunity, induced us to undertake an extensive series of experiments with proteid extracts obtained from bacterial cell masses. Some of this work is sufficiently advanced for us to record our results in part.

Hypersusceptibility may easily be induced in guinea-pigs with proteid extracts obtained from the bacterial cell. The first injection of most of the extracts used by us seems comparatively harmless to the animal. A second injection of the same extract shows, however, that profound physiological changes have taken place. A definite period must elapse between the first and second injection. The symptoms presented by the guinea-pigs as a result of the second injection resemble those caused by horse serum.

The phenomenon induced by a second injection is followed (in certain cases) by an immunity to the corresponding infection.

These results strengthen our belief that the phenomenon of hypersusceptibility has a practical significance in the

prevention and cure of certain infectious processes. It gives a possible explanation to the period of incubation of some of the communicable diseases. Is it a coincidence that the period of incubation of a number of infectious diseases is about ten to fourteen days, which corresponds significantly with the time required to sensitize animals with a strange proteid? In certain infectious diseases with short periods of incubation, such as pneumonia, the crisis which commonly appears about the tenth day may find a somewhat similar explanation. It is evident that disease processes produced by soluble toxins, such as diphtheria and tetanus, do not belong to the category now under consideration.

We propose to pursue our work along these lines so as to develop this working hypothesis into possible practical results leading to the prevention and cure of certain of the communicable diseases.

Extract of colon bacillus. — The extract from the colon bacillus in the following experiments was obtained as follows:

A two-day old culture of *B. coli communis* in Dunham's solution was used to heavily inoculate the surface of eighty-four large agar plates. These plates were grown at 37° C. for four days and the surface growth collected.

The bacterial mass was frozen forty-eight hours at about 15° F., thawed at room temperature, and then ground with sand by hand in a mortar for five hours, shaken vigorously half an hour, and again frozen eighteen hours. After again thawing, the fluid was diluted with salt solution and filtered through a Berkefeld filter. The clear filtrate gave a distinct coagulum with heat and acetic acid.

All the other extracts were obtained by a similar process. In the case of the tubercle bacillus, the bacterial mass was first washed three days in running water to eliminate the soluble tuberculin as much as possible.

No. G.P.	First Injection.	Interval in Days.	Second Injection.	Result.
817...	5 cc. colon extract, subcutaneously.	35	6 cc. colon extract, intraperitoneally.	Marked symptoms.
819...	1 cc. colon extract, subcutaneously.	35	6 cc. colon extract, intraperitoneally.	Mild symptoms.
818...	1 cc. colon extract, subcutaneously.	35	6 cc. colon extract, subcutaneously.	Marked symptoms.
820...	1 cc. colon extract, subcutaneously.	35	6 cc. colon extract, subcutaneously.	Slight symptoms.
821...	1 cc. colon extract, subcutaneously.	35	6 cc. colon extract, intraperitoneally.	Mild symptoms.
822...	.5 cc. colon extract, subcutaneously.	35	6 cc. colon extract, subcutaneously.	Mild symptoms.
823...	.1 cc. colon extract, subcutaneously.	35	6 cc. colon extract, intraperitoneally.	Slight symptoms.
824...	.01 cc. colon extract, subcutaneously.	35	6 cc. colon extract, subcutaneously.	Marked symptoms.
825...	.005 cc. colon extract, subcutaneously.	35	6 cc. colon extract, intraperitoneally.	Severe symptoms.

The hypersusceptibility induced by the colon extracts manifested itself by symptoms resembling those already described in the case of horse serum. The guinea-pigs scratched at the mouth with their hind legs. Most of them showed evidences of respiratory embarrassment by quickened, labored, or irregular breathing. Many of the pigs lay over on their sides, which is such a common symptom. A few developed jerky movements; but in no case was convulsion noted. The pigs looked quite sick and ill at ease, but gradually recovered, so that by next morning they seemed normal.

Ten days following the second injection of the extract all the above pigs were given five cubic centimeters of a heavy emulsion of colon bacillus from twenty-four-hour-old agar slants, but showed no symptoms, and remain in good condition. Three controls received the same injection and died in twelve hours.

Yeast. — The manifestations of hypersusceptibility produced by the proteid extract from yeast cells are restlessness, scratching, irregular respirations; the guinea-pigs lie down

and look sick; sometimes jerky movements are seen and, in one instance, convulsions.

No. G.P.	First Injection.	Interval in Days.	Second Injection.	Result.
755...	1 cc. extract yeast cells, subcutaneously.	22	6 cc. extract yeast cells, intraperitoneally.	Very severe symp- toms.
754...	.5 cc. extract yeast cells, subcutaneously.	22	6 cc. extract yeast cells, subcutaneously.	Slight symptoms.
864...	.1 cc. extract yeast cells, subcutaneously.	19	5 cc. extract yeast cells, subcutaneously.	Slight symptoms.
803...	1 cc. extract yeast cells, subcutaneously.	27	6 cc. extract yeast cells, intraperitoneally.	Mild symptoms.
807...	1 cc. extract yeast cells, subcutaneously.	27	6 cc. extract yeast cells, intraperitoneally.	Mild symptoms.
801...	1 cc. extract yeast cells, subcutaneously.	27	6 cc. extract yeast cells, intraperitoneally.	Marked symptoms.
809...	1 cc. extract yeast cells, subcutaneously.	27	6 cc. extract yeast cells, intraperitoneally.	Dead, 2 hrs. 10 min.
802...	1 cc. extract yeast cells, subcutaneously.	27	6 cc. extract yeast cells, subcutaneously.	Slight symptoms.
805...	1 cc. extract yeast cells, subcutaneously.	27	6 cc. extract yeast cells, subcutaneously.	Slight symptoms.
810...	1 cc. extract yeast cells, subcutaneously.	27	6 cc. extract yeast cells, subcutaneously.	Marked symptoms.
806...	1 cc. extract yeast cells, subcutaneously.	27	6 cc. extract yeast cells, subcutaneously.	Very severe symp- toms.
815...	.005 cc. extract yeast cells, subcutaneously.	27	5 cc. extract yeast cells, subcutaneously.	No symptoms.
814...	.01 cc. extract yeast cells, subcutaneously.	27	5 cc. extract yeast cells, subcutaneously.	Slight symptoms.
813...	.02 cc. extract yeast cells, subcutaneously.	27	5 cc. extract yeast cells, subcutaneously.	Mild symptoms.
812...	.1 cc. extract yeast cells, subcutaneously.	27	5 cc. extract yeast cells, subcutaneously.	Severe symptoms.
811...	.5 cc. extract yeast cells, subcutaneously.	27	5 cc. extract yeast cells, subcutaneously.	Very severe symp- toms.
801...	5 cc. extract yeast cells, subcutaneously.	27	5 cc. extract yeast cells, subcutaneously.	Mild symptoms.
800...	10 cc. extract yeast cells, subcutaneously.	27	5 cc. extract yeast cells, subcutaneously.	Very severe symp- toms.

HAY BACILLUS.

No. G.P.	First Injection.	Interval in Days.	Second Injection.	Result.
863...	1 cc. extract subtilis, subcutaneously.	14	7 cc. extract subtilis, intraperitoneally.	Slight symptoms.
864.	10 cc. extract subtilis, subcutaneously.	25	6 cc. extract subtilis, intraperitoneally.	Marked symptoms.
865...	8 cc. extract subtilis, subcutaneously.	25	6 cc. extract subtilis, intraperitoneally.	No symptoms.
866...	6 cc. extract subtilis, subcutaneously.	25	6 cc. extract subtilis, intraperitoneally.	Marked symptoms.
868...	2 cc. extract subtilis, subcutaneously.	25	6 cc. extract subtilis, intraperitoneally.	Marked symptoms.
870.	.5 cc. extract subtilis, subcutaneously.	25	6 cc. extract subtilis, intraperitoneally.	Marked symptoms.
871...	.1 cc. extract subtilis, subcutaneously.	25	6 cc. extract subtilis, intraperitoneally.	Slight symptoms.
872...	.01 cc. extract subtilis, subcutaneously.	25	6 cc. extract subtilis, intraperitoneally.	Slight symptoms.
873...	.001 cc. extract subtilis, subcutaneously.	25	6 cc. extract subtilis, intraperitoneally.	Slight symptoms.

Anthrax.—Indications of hypersusceptibility produced by anthrax are scratching, rapid respirations; pigs frequently fall over on their sides and look sick; none of the pigs coughed or had convulsions.

No. G.P.	First Injection.	Interval in Days.	Second Injection.	Result.
842...	10 cc. extract of anthrax, subcutaneously.	21	6 cc. extract of anthrax, subcutaneously.	Marked symptoms.
843...	5 cc. extract of anthrax, subcutaneously.	21	6 cc. extract of anthrax, subcutaneously.	Mild symptoms.
844...	1 cc. extract of anthrax, subcutaneously.	21	6 cc. extract of anthrax, subcutaneously.	Mild symptoms.
845...	1 cc. extract of anthrax, subcutaneously.	21	6 cc. extract of anthrax, subcutaneously.	Slight symptoms.
846...	1 cc. extract of anthrax, subcutaneously.	21	6 cc. extract of anthrax, subcutaneously.	Severe symptoms.
847...	1 cc. extract of anthrax, subcutaneously.	21	6 cc. extract of anthrax, subcutaneously.	Mild symptoms.
848...	5 cc. extract of anthrax, subcutaneously.	21	6 cc. extract of anthrax, subcutaneously.	Mild symptoms.
849...	1 cc. extract of anthrax, subcutaneously.	21	6 cc. extract of anthrax, subcutaneously.	Slight symptoms.
850...	.01 cc. extract of anthrax, subcutaneously.	21	6 cc. extract of anthrax, subcutaneously.	Slight symptoms.
851...	.005 cc. extract of anthrax, subcutaneously.	21	6 cc. extract of anthrax, subcutaneously.	Slight symptoms.
852...	1 cc. extract of anthrax, subcutaneously, daily, 7 days.	11	6 cc. extract of anthrax, subcutaneously.	No symptoms.
853...	1 cc. extract of anthrax, subcutaneously, daily, 7 days.	11	6 cc. extract of anthrax, subcutaneously.	No symptoms.
854...	1 cc. extract of anthrax, subcutaneously, daily, 7 days.	11	4 cc. extract of anthrax, subcutaneously.	Mild symptoms.
855...	1 cc. extract of anthrax, subcutaneously, daily, 7 days.	11	6 cc. extract of anthrax, subcutaneously.	Slight symptoms.
856...	1 cc. extract of anthrax, subcutaneously, daily, 7 days.	11	6 cc. extract of anthrax, subcutaneously.	No symptoms.

All the above guinea-pigs were subsequently inoculated with a virulent culture of anthrax. They all died in a few days with the usual lesions.

A number of guinea-pigs were given the extract from anthrax bacilli before infection; some were given a single injection, some two injections, and others daily injections for twenty days. Other guinea-pigs were given the extract used as a vaccine, both in single and repeated injections, after being infected with anthrax bacilli. The extract did not seem to have any influence on the course of the disease, whether given before or after the infection.

Tuberculosis.—The indications of hypersusceptibility induced by extract of tubercle bacilli are restlessness, scratching, irregular respiration, tremor; most of the pigs lie down on their sides and look sick.

No. G.P.	First Injection.	Interval in Days.	Second Injection.	Result.
827...	.1 cc. extract human tubercle bacilli, subcutaneously.	32	6 cc. extract human tubercle bacilli, subcutaneously.	Mild symptoms.
828...	1 cc. extract human tubercle bacilli, subcutaneously.	32	6 cc. extract human tubercle bacilli, subcutaneously.	No symptoms.
829...	2 cc. extract human tubercle bacilli, subcutaneously.	32	6 cc. extract human tubercle bacilli, subcutaneously.	Mild symptoms.
830...	3 cc. extract human tubercle bacilli, subcutaneously.	32	6 cc. extract human tubercle bacilli, subcutaneously.	Mild symptoms.
831...	4 cc. extract human tubercle bacilli, subcutaneously.	32	6 cc. extract human tubercle bacilli, subcutaneously.	Mild symptoms.
832...	5 cc. extract human tubercle bacilli, subcutaneously.	32	6 cc. extract human tubercle bacilli, intraperitoneally.	Severe symptoms.
833...	6 cc. extract human tubercle bacilli, subcutaneously.	32	6 cc. extract human tubercle bacilli, intraperitoneally.	Slight symptoms.
834...	7 cc. extract human tubercle bacilli, subcutaneously.	32	10 cc. extract human tubercle bacilli, intraperitoneally.	Slight symptoms.
835...	8 cc. extract human tubercle bacilli, subcutaneously.	32	6 cc. extract human tubercle bacilli, intraperitoneally.	Mild symptoms.
836...	9 cc. extract human tubercle bacilli, subcutaneously.	32	6 cc. extract human tubercle bacilli, subcutaneously.	No symptoms.

The guinea-pigs which have reacted to two injections of proteid extract obtained from the tubercle bacillus are now being tested for immunity to infection with tubercle cultures.

Typhoid.—The indications of hypersusceptibility induced by two injections of typhoid extract manifest themselves by rapid respirations; most of the pigs lie down on their sides. The symptoms presented by this series of pigs were mild.

No. G.P.	First Injection.	Interval in Days.	Second Injection.	Result.
857...	10 cc. typhoid extract, subcutaneously.	34	10 cc. typhoid extract, subcutaneously.	Slight symptoms.
858...	5 cc. typhoid extract, subcutaneously.	34	10 cc. typhoid extract, subcutaneously.	Slight symptoms.
859...	1 cc. typhoid extract, subcutaneously.	34	10 cc. typhoid extract, subcutaneously.	Slight symptoms.
860...	1 cc. typhoid extract, subcutaneously.	34	6 cc. typhoid extract, intraperitoneally.	Slight symptoms.
862...	.5 cc. typhoid extract, subcutaneously.	34	10 cc. typhoid extract, subcutaneously.	Slight symptoms.
863.	.1 cc. typhoid extract, subcutaneously.	34	10 cc. typhoid extract, subcutaneously.	Slight symptoms.

Nine days following the second injection of the extract five pigs of the above series which had received ten cubic centimeters of the typhoid extract at the second injection resisted a large dose of a virulent typhoid culture. Two controls died in eighteen hours. One or two of the pigs which had received the extract were slightly sick the following day, but the next day had fully recovered and have remained so. A definite immunity was, therefore, conferred by the two injections of extract from the typhoid bacillus.

SUMMARY AND CONCLUSIONS.

Profound changes, perhaps, in the central nervous system, are probably produced by the first injection of a strange proteid.

Guinea-pigs may be sensitized with horse serum by injections directly into the heart. From this it appears that the cells lining the peritoneal cavity or the connective tissue cells of the subcutaneous tissue do not necessarily play a part in the phenomenon of hypersusceptibility.

Guinea-pigs may be sensitized with the filtrate obtained from horse serum after precipitation with ammonium sulphate.

Formaldehyd does not appreciably influence the toxicity of horse serum and has no effect upon the sensitizing action.

The sensitizing substance is not dialyzable through a collodion sack.

The toxic principle is not altered by various ferments such as taka-diastase, pancreatin, rennin, myrosin, invertin, emulsin, pepsin, ingluvin, malt, or papain, nor by certain alkaloids such as atropin, strychnin, morphin, or caffein; it is also not altered by calcium chlorid, sodium nitrate, sodium chlorid, magnesium sulphate, or ammonium sulphate.

Guinea-pigs sensitized with horse serum do not react to the second injection of other proteid substances such as peptone, extract of peas, egg albumen, and milk. Conversely, guinea-pigs sensitized with subcutaneous injections of these substances do not react to a subsequent injection of horse serum.

Guinea-pigs show quite as high a degree of susceptibility to cattle, sheep, hog, dog, and cat serum as they do to horse serum.

Guinea-pigs are quite susceptible to injections of hemoglobin, egg albumen, milk, or the extract of peas when given two injections with an interval of at least ten days. Simpler albuminous substances, such as peptone, seem to have slight sensitizing and poisonous properties, while lower nitrogenous compounds such as leucin and tyrosin possess none at all.

The reaction following a second injection of proteid matter in the guinea-pig appears to be common to all the higher forms of albuminous substances, no matter from what source.

This phenomenon of hypersusceptibility in the guinea-pig may be useful as a physiological test to distinguish true proteid substances from the lower forms of nitrogenous compounds.

The refined antitoxic serum, bulk for bulk, is quite as toxic to sensitized guinea-pigs as the untreated serum from which it was precipitated and dialyzed. There is, however, a distinct advantage gained in using the concentrated serum, as the same number of units may be given in half the bulk, and it is well known that the serum reaction in man depends partly upon the quantity of serum given.

Serum from one horse appears quite as toxic as serum from other horses. The apparent differences seem to depend upon something connected with the sensitizing action.

The immunity produced by repeated injections, termed "anti-anaphylaxis" by Besredka and Steinhardt, appears to be relatively not quite as lasting and definite as many instances of active immunity against bacterial infections.

Hypersusceptibility to the toxic action of horse serum is transmitted through the female guinea-pig; the male has no influence.

The susceptibility is not transmitted through the milk.

Maternal transmission of hypersusceptibility succeeds whether the female guinea-pig is sensitized before or after conception.

The phenomenon of hypersusceptibility appears to have no relation to aggressins.

Methemaglobin is not present in the blood of guinea-pigs dead of a second injection of horse serum.

Oxygen has no influence upon the symptoms.

Neither diphtheria toxine nor tetanus toxine appreciably influences the phenomenon of hypersusceptibility produced by horse serum.

The removal of the spleen or thyroid gland does not influence hypersusceptibility in the guinea-pig.

Guinea-pigs fed upon beef are susceptible to a subsequent injection of cattle serum.

Guinea-pigs fed with cooked meat are not susceptible to subsequent injections of serum.

When a second injection of horse serum is given directly into the heart of a susceptible guinea-pig the symptoms are manifested with promptness and virulence. Under these circumstances, .01 cubic centimeter injected directly into the heart in one instance was sufficient to cause the death of a sensitized guinea-pig.

Guinea-pigs remain susceptible a very long time. There is no diminution in the susceptibility of a guinea-pig to subsequent injections of horse serum for at least one year. The longest period we have observed is four hundred and eighty days.

We have never seen symptoms resulting from the first injection of horse serum in a guinea-pig born of an untreated mother.

The problem of hypersusceptibility has an important bearing upon the question of immunity. Our work indicates that hypersusceptibility is either an essential element or one stage in the process of resistance to a certain class of diseases. We cannot escape the conviction that further studies upon the phenomenon of hypersusceptibility will have an important bearing upon the prevention and cure of certain infectious processes. The hypersusceptibility obtained by bacterial proteids and the subsequent immunity furnish data for this belief.

From our work upon the proteid substances of animal and vegetable origin, it was but a step to the albuminous content of the bacterial cell. Experimental studies upon the bacterial proteids are of the greatest importance on account of the practical uses to which results along this line may lead.

Hypersusceptibility may easily be induced in guinea-pigs with proteid extracts obtained from the bacterial cell. The first injection of the extracts used by us seems comparatively harmless to the animal. A second injection of the same extract shows, however, that profound physiological changes have taken place. A definite period must elapse between the first and the second injection. The symptoms presented by the guinea-pig as a result of the second injection resemble those caused by horse serum.

The phenomenon induced by a second injection is followed, in certain cases, by an immunity to the corresponding infection.

These results give a possible explanation of the period of incubation in some of the communicable diseases. Is it a coincidence that the period of incubation in a number of infectious diseases is about ten to fourteen days, which corresponds significantly with the time required to sensitize animals with a strange proteid? In certain infectious diseases with a short period of incubation, such as pneumonia, the crisis, which commonly appears in about ten days, may be similarly explained. It is evident that diseased processes produced by soluble toxins, such as diphtheria and tetanus, do not belong to the category now under consideration.

The phenomenon of hypersusceptibility has been produced in the guinea-pig by extracts obtained from the colon bacillus, yeast, hay bacillus, anthrax, tubercle bacillus, and the typhoid bacillus. The hypersusceptibility produced by the colon and typhoid bacillus was followed by a definite immunity to the corresponding infections. In the case of anthrax, however, immunity does not follow hypersusceptibility to the anthrax proteid. We are, therefore, not dealing with a general law applicable to all infections, but with certain limitations as in the case of antitoxic immunity. We are now applying this principle to tuberculosis, plague, cholera, and other infections.

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EFFECTS OF EXPERIMENTAL INJURIES OF THE PANCREAS.*

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Since Balser's¹ publication in 1882 on fat necrosis, which first indicated the close relationship between this peculiar, frequently fatal disease and acute inflammation of the pancreas, much work was done in the effort to produce fat necrosis through some experimental injury of the pancreas.

The experiments reported may be divided in two large groups. In the first group different substances were injected into the pancreatic duct or into the body of the gland. To this group of experiments belong the researches of Flexner,² Opie,³ and more recently Hess,⁴ Eppinger,⁵ Polya,⁶ and Guleke.⁷ The best results seemed to have been obtained with injection of oil and bile.

In the second group of publications the body of the pancreas was mechanically injured in different ways. Hildebrand⁸ was the first to experiment along these lines. He either doubly ligated and severed the pancreas, producing a stasis of secretion, or ligated most of the blood vessels leading to and from the pancreas, and produced a stasis of circulation, or cut the pancreatic duct allowing a free exit of the pancreatic juice in the peritoneum. Williams⁹ operated in a similar way. Katz and Winkler¹⁰ ligated the pancreas in several places, and produced both necrosis of the pancreas and fat necrosis.

Very recently, Doberauer¹¹ reported a series of twenty-one experiments on dogs. In each case he doubly ligated and severed the pancreas with identical results, viz.: the development of fat necrosis, subserous peritoneal hemorrhages and free hemorrhagic fluid in the peritoneum. The animals were either dead or moribund within twenty-four hours. The author ascribes the fatal results in his experiments

*Received for publication May 16, 1907.

to a combination of stasis of secretion, some abnormality in the circulation, and a lesion of the parenchyma of the pancreas.

These experiments show clearly that injuries of the pancreas produce different effects on the organism from the complete removal of the organ. After the latter operation the animal succumbs with the symptoms of subacute diabetes, while a comparatively slight injury to the organ may kill it within twenty-four hours, producing an entirely different symptom — complex.

But with all that, it is very difficult to form a correct idea of the etiological relation between a certain injury to the pancreas and the disease process that so rapidly kills the animal, because in all the experimental work thus far reported, an injury which results fatally in a certain number of animals produces no effects on others.

The experiments of Doberauer differ from all previous investigations in the fact that he obtained the same results in every experiment.

On the one hand it seemed advisable to repeat Doberauer's experiments, because, if found correct, they could subsequently be varied so as to afford a clearer insight into the etiological moment of the injury, which produced the acute fatal disease of the animal.

On the other hand a mechanical injury of pancreas is generally a less complicated experiment than the injection of different substances in the pancreatic duct, as the latter may reach the general circulation and thus injure the organism.

For this reason a series of experiments was undertaken in which the pancreas of dogs and cats was injured mechanically in different ways and the effect on the organism studied. Following is the detailed description of the experiments:

1. *Double ligation and section of the pancreas.*

Dog No. 1, X., 14.06. Laparotomy. The pancreas was doubly ligated and severed at its middle. The animal was apparently well after the operation. Ten days later he was killed. At the autopsy no abnormality was found, with the exception of the parts of the pancreas near the ligatures, which were in a state of interstitial pancreatitis.

Dog No. 2, X., 16.06. Laparotomy. Double ligation and section of the pancreas. The animal recovered after the operation and was well until killed two weeks later. The autopsy showed the same condition as in the Dog No. 1.

Dog No. 3, X., 18.06. Laparotomy. Double ligation and section of the pancreas. The animal died in twenty-four hours. The autopsy showed congestion of the pancreas near the ligatures (otherwise the organ was macroscopically normal), sero-fibrinous peritonitis, and no fat necrosis.

Dog No. 4, X., 22.06. Laparotomy. Double ligation and section of the pancreas. The animal recovered after the operation and was well until killed ten days later. The autopsy showed the same condition as in the Dog No. 1.

Dog No. 5, X., 25.06. Laparotomy. Double ligation and section of the pancreas. Animal was well until killed three weeks after the operation. The autopsy showed the same condition as in the Dog No. 1.

Dog No. 6, X., 29.06. Laparotomy. Double ligation and section of the pancreas. Animal was well until killed ten days after the operation. The autopsy showed the same condition as in the Dog No. 1.

In this series of experiments the injury to the pancreas consisted in a stasis of the secretion of the organ, or else the pancreatic juice, lacking free access to the duodenum, was absorbed by the lymph or blood vessels. The latter explanation may be more plausible, as there was no change in the condition of the gland, with the exception of the segments in the immediate vicinity of the ligatures. Such an injury of the pancreas apparently produces no ill effect on the organism. The only animal that died twenty-four hours after the operation was probably infected.

II. *Mechanical crushing of a part of the pancreas.*

Dog No. 7, XI.1. Laparotomy. A part of the pancreas about one inch long at the pancreatic duct was crushed with an artery forceps and every bleeding vessel ligated separately. The animal recovered after the operation and was well until killed two weeks later. The autopsy showed the crushed part of the pancreas to be partly absorbed and partly in a state of interstitial degeneration. The rest of the pancreas was normal.

Dog No. 8, XI.3. Laparotomy. Crushing of a part of the pancreas. The animal remained well and was killed sixteen days after the operation. The autopsy showed the same condition as in the Dog No. 7.

Dog No. 9, XI.6. Laparotomy. Crushing of a part of the pancreas. The animal was well and was killed three weeks after the operation. The autopsy showed the same condition as in the Dog No. 7.

Dog No. 10, XI.12. Laparotomy. Crushing of a part of the pancreas.

The animal was well and was killed five weeks after the operation. The autopsy showed the same condition as in the Dog No. 7.

Cat No. 1, XI.8. Laparotomy. Crushing of a part of the pancreas. The animal was well and was killed sixteen days after the operation. The autopsy showed the same condition as in the Dog No. 7.

Cat No. 2, XI.10. Laparotomy. Crushing of a part of the pancreas. The animal was well and was killed three weeks after the operation. The autopsy showed the same condition as in the Dog No. 7.

Cat No. 3, XI.15. Laparotomy. Crushing of a part of the pancreas. The animal was well and was killed two weeks after the operation. The autopsy showed the same condition as in the Dog No. 7.

In these experiments a part of the parenchyma of the organ is injured, and the normal pancreatic juice is given a free exit in the peritoneum. There seemed to be no ill effect from it on the organism.

III. *Crushing or section of the pancreas and ligation of the pancreatic blood vessels.*

Dog No. 11, XI.17. Laparotomy. A part of the pancreas was crushed and the most important blood vessels were ligated. The animal recovered after the operation, but was losing weight. It was killed twelve days after the operation. The autopsy showed interstitial pancreatitis, but no other abnormality.

Dog No. 12, XI.19. Laparotomy. A part of the pancreas was crushed and the blood vessels were ligated. The animal died in twenty-four hours. The autopsy showed acute pancreatitis, free hemorrhagic fluid in the peritoneum, and fat necrosis.

Dog No. 13, XI.21. Laparotomy. Double ligation and section of the pancreas, ligation of blood vessels. The animal died in about forty hours. The autopsy showed acute pancreatitis, free hemorrhagic fluid in the peritoneum, and fat necrosis.

Dog No. 14, XI.23. Laparotomy. Double ligation and section of the pancreas, ligation of blood vessels. The animal recovered after the operation. It was killed ten days later. The autopsy showed interstitial pancreatitis.

Dog No. 15, XI.26. Laparotomy. A part of the pancreas was crushed and the blood vessels were ligated. The animal died in forty-eight hours. The autopsy showed acute pancreatitis, free hemorrhagic fluid in the peritoneum, and fat necrosis.

Dog No. 16, XI.28. Laparotomy. A part of the pancreas was crushed and the blood vessels were ligated. The animal recovered after the operation, but was emaciating. It was killed three weeks after the operation. The autopsy showed interstitial pancreatitis.

Cat No. 4, XI.30. Laparotomy. A part of the pancreas was crushed

and the blood vessels were ligated. The animal recovered. It was killed two weeks after the operation, showing interstitial pancreatitis.

Cat No. 5, XII.1. Laparotomy. A part of the pancreas was crushed and blood vessels were ligated. The animal recovered. It was killed ten days after the operation and showed interstitial pancreatitis.

In this series of experiments some abnormality in the blood supply was produced in addition to the results received in the previous experiments. In every experiment of this series the pancreas was found diseased, but while in some cases the operation was fatal to the animal, in others again the organism as a whole seemed to suffer but little.

IV. *Multiple ligation of the pancreas.*

Dog No. 17, XII.10. Laparotomy. Six ligatures were placed around different parts of the pancreas, neither section nor crushing were done. The animal appeared quite sick after the operation and was killed three days later. The autopsy showed acute pancreatitis, free hemorrhagic fluid in the peritoneum, no fat necrosis.

Dog No. 18, XII.13. Laparotomy. Eight ligatures were placed around the pancreas. The animal died forty-eight hours after the operation. The autopsy revealed acute pancreatitis, free hemorrhagic fluid in the peritoneum, subserous hemorrhages, and fat necrosis.

Dog No. 19, XII.17. Laparotomy. Six ligatures were placed around the pancreas. The animal recovered but was losing flesh. It was killed three weeks after the operation. The autopsy showed chronic interstitial pancreatitis but no other abnormality.

Dog No. 20, XII.20. Laparotomy. Seven ligatures were placed around the pancreas. The animal died twenty-four hours after the operation. The autopsy showed gangrene of the pancreas, gangrene of the duodenum and peritonitis but no fat necrosis.

Dog No. 21, XII.28. Laparotomy. Six ligatures were placed around the pancreas. The animal recovered. It was killed ten days after the operation. The autopsy showed chronic interstitial pancreatitis but no other abnormality.

Dog No. 22, I.07.3. Laparotomy. Six ligatures were placed around the pancreas. The animal appeared very sick and was killed thirty-six hours after the operation. The autopsy showed acute pancreatitis, free hemorrhagic fluid in the peritoneum, subserous hemorrhages, and fat necrosis.

Dog No. 23, I.8. Laparotomy. Eight ligatures were placed around the pancreas. The animal died in twenty-four hours. The autopsy showed gangrene of the pancreas, duodenum greatly congested, peritonitis, free hemorrhagic fluid in the peritoneum, subserous hemorrhages, and fat necrosis.

Cat No. 6, I.15. Laparotomy. Six ligatures were placed around the pancreas. The animal died in sixteen hours. The autopsy showed pancreas congested, some free hemorrhagic fluid in the peritoneum and no other apparent cause of death.

Cat No. 7, I.24. Laparotomy. Seven ligatures were placed around the pancreas. The animal looked sickly. It was killed one week after the operation. The autopsy showed chronic interstitial pancreatitis.

Cat No. 8, II.5. Laparotomy. Seven ligatures were placed around the pancreas. The animal recovered and was killed three weeks after the operation. The autopsy showed interstitial pancreatitis.

Cat No. 9, II.12. Laparotomy. Seven ligatures were placed around the pancreas. The animal died twenty-four hours after the operation. The autopsy showed acute pancreatitis, free hemorrhagic fluid in the peritoneum, subserous hemorrhages, fat necrosis.

In this last series of experiments the injury of the pancreas was severer than in any of the previous experiments. A stasis nearly complete of both secretion and circulation was produced. Still even such an injury of the pancreas is not always fatal to the organism.

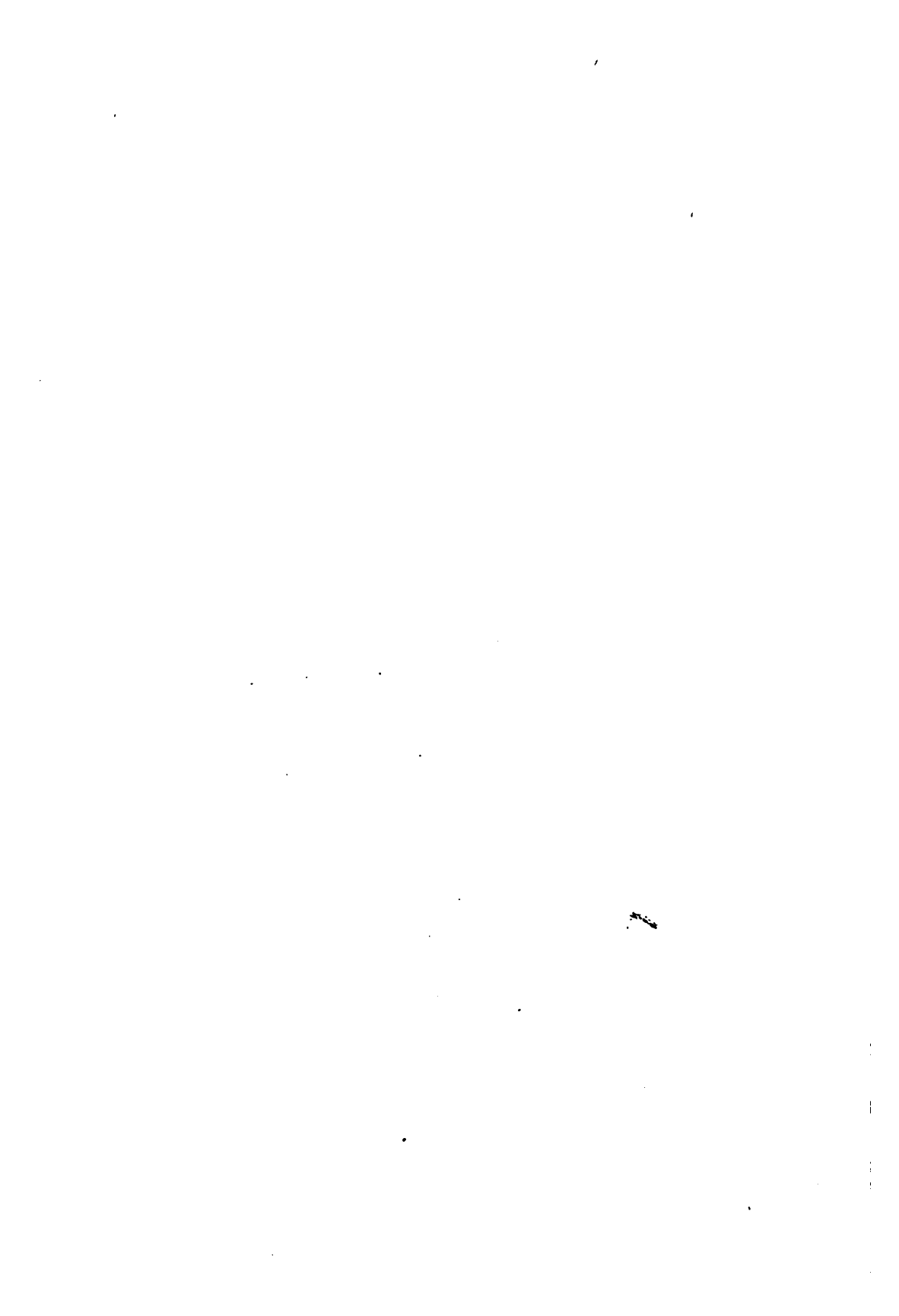
The analysis of all these experiments indicates that those injuries of the pancreas produce the gravest effects on the organism which cause the most serious interference with the circulation of the organ, as in Series III. and IV. To produce a fatal disease it does not suffice to interfere partly with the free secretion of the pancreatic juice into the intestines as in the first series of experiments, or to injure some of the parenchyma, at the same time allowing the juice to secrete into the peritoneal cavity as in Series II. The interference with the circulation must be such as to produce a lesion of the whole organ so that not only will the organism be deprived of the normal function of the pancreatic cells, as after extirpation of the organ, but also every cell will become diseased and begin to act abnormally and injuriously to the organism. If an animal remains apparently healthy even after six or more ligatures were placed around the pancreas, then some of the gland parenchyma must have remained normal functionally. Peritoneal infection which frequently occurs in these experiments need not always be caused by extraneous infection, it may be produced by stasis of secretion in the pancreas and subsequent invasion

of pathogenic bacteria from the duodenum through the pancreatic duct. The infection again aggravates the effect of the pancreatic injury upon the organism. It is also very important to note that very frequently animals which succumb from the effects of the injury of the pancreas do not show any fat necrosis and that fat necrosis is not a necessary sequence of a severe injury of the pancreas, though this does not prove that fat necrosis, when present, may not be due to a lesion of the pancreas and to no other cause.

[Before I close I deem it a very pleasant duty to express here my gratitude to Dr. T. Mitchell Prudden for the great interest he showed in my work.]

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THE NORMAL PULSATIONS WITHIN THE ESOPHAGUS.*

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Our knowledge of the cardiac arrhythmias has been much advanced within recent years through the study of graphic records. For this reason, every new application of this method is a welcome addition to our means for investigating heart disease. Minkowski^{1,2} has recently shown that the movements of the left auricle can be recorded from within the esophagus. He introduces a stomach tube over the end of which a small rubber balloon has been fastened. The balloon is inflated at any desired point of the esophagus and the changes in pressure exerted upon it by surrounding structures is transmitted to the exterior through the stomach tube and may be recorded by any convenient system of tambours. The esophageal cardiograms thus obtained indicate the contractions of both auricles and ventricles.

During the past six months we have made a number of such cardiograms mostly from patients with heart disease. On account of the difficulty in the interpretation of such pathological tracings it seemed advisable to us to publish an exceptionally good set of normal esophageal tracings obtained from one of ourselves. We are all the more led to do so for the reason that these tracings differ in some important particulars from those published as normal by Minkowski.

Technic.—The technic followed was practically that advised by Minkowski. The pharynx was cocaineized and a small-sized stomach tube was then passed into the esophagus to any desired depth. The lower end of this tube was covered with a small rubber balloon; the upper end was connected by means of a Y-tube with the recording lever of a Jaquet cardiosphmygmograph and with a bulb that could

* Received for publication May 17, 1907.

be used to blow air into the system and so inflate the rubber balloon. The time relations on the curves were obtained by comparison with radial tracings.

It was found that good tracings are more readily obtained from thin than from fat individuals. Respirations affect the character of the tracings very considerably and it was usually necessary to take the tracings at a particular point in the respiratory cycle or even during the act of slow inspiration. Esophageal tracings demand considerably more from the patient than does the ordinary passage of a stomach tube; for they consume more time and it is absolutely necessary to avoid gagging. When to this is added the fact that our patients are suffering from heart disease, that the tube often increases the irregularity of their hearts and may cause dyspnea, cyanosis, etc., it will be seen that this is not a method of examination that is applicable to every patient. However, in many instances, after the first discomfort of passing the tube is over, the patient lies quietly for an indefinite period of time with the tube in position and it is only necessary to keep his mouth free of mucus.

Normal tracings. — It was found that the esophageal cardiograms from a single individual vary according to the point of esophagus from which they are taken, just as the ordinary cardiogram differs according to the point of the chest whence it is obtained. In order to compare the curves obtained at different levels, they have been redrawn to a time scale and have been arranged one above the other, each having as a fixed point the beginning of the radial pulse. Minor inaccuracies in such a scheme naturally occur for the reason that not all systoles have the same duration; but in view of the fact that the time of systole is comparatively constant with slight variations of heart rate, these inaccuracies are of but little importance compared to the advantages of such a schematic arrangement.

In Fig. 1, the uppermost curve *a* represents the radial pulse, the next, *b*, the apex beat, and the third, *c*, the jugular tracing. Tracing *d* was obtained within the esophagus,

thirty centimeters from the line of the teeth, and apparently represents the pulsations of the transverse aorta, for it corresponds in time to the carotid wave on the jugular pulse, except that it is inverted. This indicates that the transverse aorta pulls away from the esophagus at each beat.

The four next tracings, *e*, *f*, *g*, *h* (see also Figs. 2, 3, and 4), were obtained at a distance of thirty-four to thirty-eight centimeters from the teeth, evidently at about the level of the seventh dorsal vertebra, at which location according to Braune's Atlas³ the anterior surface of the esophagus lies in contact with the left auricle. In all of these tracings the systole of the auricle is plainly indicated by the sharp descent of the curves at the line I., the duration of this descent being about .06 second. The beginning of the ventricular contraction as determined from the apex beat is indicated by line II. It will be seen that there is a rise in all the esophageal cardiograms coincident with or a little before the beginning of ventricular systole. On the uppermost tracing *e* (see also Fig. 2) this soon reaches the former level and remains there except for a slight positive wave in the neighborhood of line IV. In the two lower cardiograms *g* and *h* (see also Fig. 3) this line is immediately interrupted by a negative wave which has its apex downward and which reaches its maximum a little before line III. The latter represents the time of opening of the semilunar valves and was determined from the carotid wave on the jugular tracing by allowing .02 second for the time of transmission from the heart to the neck. A precisely similar wave is seen on Erlanger's tracings taken over the auricles in a patient whose bony chest wall in this region was deficient.⁴ Erlanger holds that this is due to the diastole of the auricle. We believe on the contrary that it depends in some manner upon the ventricular contraction and for the following reasons: (1) it is far more marked on those cardiograms which were taken low down in the esophagus and consequently closer to the ventricle; (2) it is present in a case of mitral insufficiency where no evidence of auricular contraction could be seen on the esophageal tracing and where

consequently auricular diastole could hardly have produced such a well-marked wave (Fig. 5). Possibly it is due to a drawing down of the segments of the mitral valve; for, as Roy and Adami have shown, the papillary muscles do not begin to shorten until some time after the beginning of ventricular systole.¹²

After the completion of this negative wave at about line III., the esophageal cardiogram pursues a more or less even course until the end of systole approaches, when there is a rise followed by a marked fall in the tracing, best seen in *h*. This fall is also very pronounced in Erlanger's tracings over the exposed auricle and it is quite certainly due to the emptying of the auricles into the ventricles immediately after the opening of the auriculo-ventricular valves in the early part of diastole. Line IV., drawn at the beginning of this fall, may be taken, therefore, to represent the opening of these valves. Following this fall there is a more or less gradual rise to the level preceding line I., and so the cycle is completed.

The final curve *i* on our scheme shows two marked falls, one a little after line III., and one at line IV. The latter evidently corresponds to the opening of the auriculo-ventricular valves. We do not feel satisfied as to the cause of the former, though we suspect that it is due to the pulsation of the descending aorta, which at this point may pull away from the esophagus and so cause a negative wave.

The *v* wave on the jugular pulse.—Even on cursory examination of Fig. 1, one is struck by the general resemblance that exists between the *v* wave on the venous tracing and the final undulations at line IV. on the lower esophageal tracings *g*, *h*, and *i*. The main difference between them is one of time, for the positive wave on the esophageal cardiogram precedes the *v* in the jugular wave by about .10 second. We believe that both have the same cause, though originating in different auricles, and that the difference in time is due to the slow propagation of the wave from its point of origin to the veins in the neck. Two main facts support this view. In the first place, we have determined on a patient

with tricuspid insufficiency the rate of propagation of venous waves by comparing tracings taken at different levels of the neck and found this rate to be 1.2 meters per second. Assuming that this rate holds in the present instance and that the distance from the auriculo-ventricular junction to the supra-clavicular fossa is twelve centimeters, then the theoretical time lost in the passage of the wave from the one place to the other would be .10 second, which agrees almost exactly with the difference in time between the final waves in the esophageal cardiogram *k* and the *v* wave on the jugular pulse. The other reason for believing that there is considerable time lost in the passage of venous waves from the auriculo-ventricular junction to the neck is seen by inspection of Fig. 5. This was taken from a patient with mitral and tricuspid insufficiency. The entire esophageal tracing practically duplicates the jugular tracing except that the latter follows the former by about .13 second. We believe, therefore, that the *v* wave on the normal jugular pulse corresponds to the final elevation on the esophageal cardiogram and that the explanation for the one is the explanation for the other.

Curiously enough the time taken in the transmission of this wave to the neck seems to have been disregarded by most authors and Wenckebach, for example, in a recent elaborate discussion of the subject,⁵ takes no cognizance of this fact. He is thus led into the error of assuming that the *v* wave on the jugular tracing is entirely diastolic in origin and that it does not begin until the closure of the semilunar valves. When a wave appears earlier than that, he regards it as pathological and due to a stasis of blood in the auricle. In the present instance there was absolutely no reason to believe that the individual from whom the tracings were taken was in any way pathological. In Fig. 3 it will be seen that the wave begins sharply .10 to .15 second before the opening of the auriculo-ventricular valves. It thus precedes ventricular relaxation, for Erlanger estimates that the entire relaxation, which continues somewhat after the opening of the valves, lasts only about .06 second. From this as well as from comparison with a number of apex tracings (see

Fig. 1) we are led to conclude that the wave begins in the lattermost part of ventricular systole. Its sharp character and the fact that it is so much more pronounced low down in the neighborhood of the ventricle lead us to believe that it is due to some backward and upward movement of the auriculo-ventricular junction at the end of systole. Porter⁶ has shown in a dog that there is a sudden increase in intra-auricular pressure at the end of ventricular systole, and he attributes this in part to an upward movement of the mitral valves, due, as Roy and Adami have shown, to the earlier relaxation of the papillary muscles.¹² This final wave on the esophageal cardiogram terminates, as we have seen, with the fall produced by the opening of the valves leading into the ventricles.

Pathological esophageal cardiograms. — It is not our purpose to attempt a full discussion of the pathological changes in the esophageal cardiogram. Their interpretation presents very considerable difficulties, and we have several for which at present we have no adequate explanation. A number of pathological changes, however, have been described. For example, Joachim,⁷ Rautenberg,⁸ and Schreiber⁹ have shown by means of esophageal cardiograms that in Adams-Stokes' disease the left auricle contracts simultaneously with the right and independently of the ventricles. More recently Minkowski² has published esophageal tracings of extrasystoles, diminished conductivity, and diminished ventricular contractility, though it appears to us that the interpretation of his figures is not beyond question. What he publishes as a tracing from a case of mitral stenosis and insufficiency agrees in its main features so closely with tracing *c* of Fig. 1 that we should certainly doubt the advisability of drawing any binding conclusions from a tracing of this character.

In Fig. 5 is seen the esophageal, jugular, and radial tracings from a patient with mitral and tricuspid incompetency in a stage of broken compensation. It will be seen that the esophageal cardiogram coincides with the jugular tracing in

most of its details, except that the former precedes the latter by about .13 second. Neither in this nor in any other of a great number of tracings from this patient was there any graphic evidence of left auricular contractions. Apparently the auricle had ceased to contract owing to the long-continued overdistention. We have thus a condition analogous to that already described by Mackenzie¹⁰ for the right auricle; viz., a paralysis of the auricle from overdistention. The pressure within the left auricle rises during systole and falls on the opening of the mitral valve. When the ventricular contractions follow one another very rapidly, the period of heart pause is so short that it is represented by a sharp and deep negative wave. When a somewhat longer period intervenes between the ventricular contractions, the pressure within the left auricle gradually rises during the heart pause and in this way an ill-defined positive wave of stasis is produced.

Mackenzie¹¹ interprets the negative wave that immediately follows the first systolic elevation in a venous pulse, such as this, as due to the diastole of the auricle, and he argues from this that the cardiac rhythm has begun at the auriculo-ventricular bundle of His and that the auricles and ventricles are beating simultaneously. We have already seen, however, that a similar negative wave is present on normal esophageal tracings and that it seems to be produced by some movement of the ventricle. In view of this fact and of the absence of an auricular contraction wave on this tracing, we should hesitate to accept Mackenzie's interpretation of venous pulses of this character.

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DESCRIPTION OF PLATE XXI

FIG. 1. — Schematic arrangement of tracings: — *a*, radial pulse; *b*, apex beat; *c*, jugular tracing; *d*, esophageal tracing 30 centimeters from teeth; *e*, 34.5 centimeters; *f*, 35 centimeters; *g* and *h*, 37.5 centimeters; *i*, 38.5 centimeters from teeth. Line I. Beginning of auricular systole. Line II. Beginning of ventricular systole. Line III. Opening of semi-lunar valves. Line IV. Opening of auriculo-ventricular valves.

FIG. 2. — Esophageal tracing taken 34.5 centimeters from the teeth, corresponding to tracing *e* of Fig. 1.

FIG. 3. — Esophageal tracing taken 37.5 centimeters from the teeth, corresponding to tracings *g* and *h* of Fig. 1.

FIG. 4. — Simultaneous tracings from the jugular vein (above), the esophagus at thirty-eight centimeters from the teeth (middle), and the radial (below).

FIG. 5. — Tracings from a case of mitral and tricuspid insufficiency. The upper is from the esophagus, thirty-seven centimeters from the teeth, the next from the jugular vein, and the lower the radial pulse.

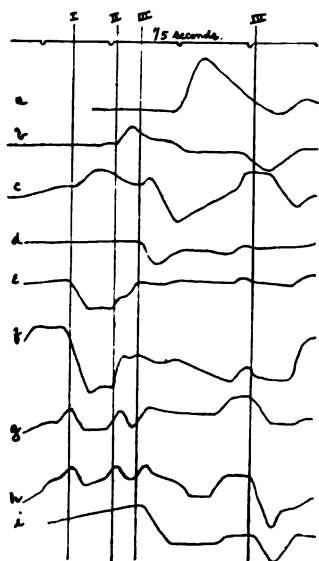


FIG. 1.

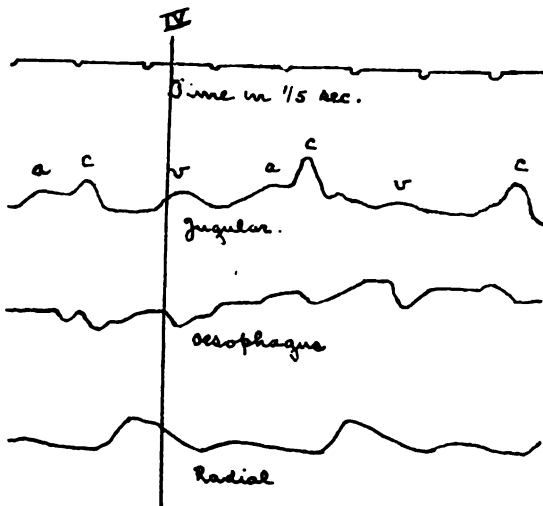


FIG. 4.

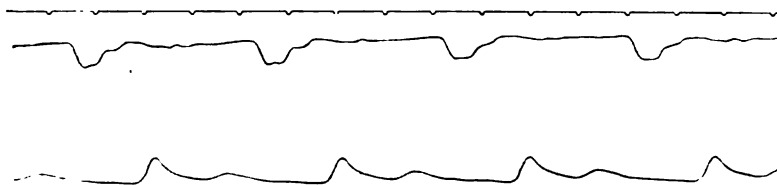


FIG. 2.

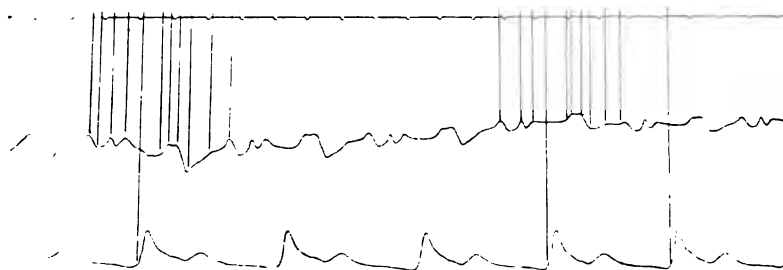


FIG. 3.

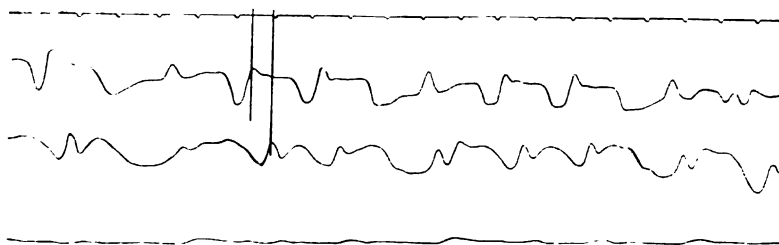


FIG. 5.

STUDIES IN MAMMALIAN TUBERCLE BACILLI, IV.
BACILLI RESEMBLING THE BOVINE TYPE FROM FOUR CASES
IN MAN.*

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The literature concerning itself with the interrelation of bovine and human tuberculosis as determined by a study of the tubercle bacilli isolated from cases of disease has grown to large proportions. Almost all observers are now agreed that there exist certain differences between human and bovine bacilli. They are, however, not agreed upon the interpretation to be placed upon these differences. The time is not yet ripe for a critical review of the various interpretations. There are needed a larger amount of statistical material concerning cultures carefully studied, and more investigations upon the possible modifications of bovine and human bacilli in other host species. In the meantime, the most reasonable attitude, and one not inimical to the public health, is one that regards all bacilli resembling the bovine type which are found in man as bovine in origin and as legitimate causes of the tubercular processes found. Attention should, however, be called to the possibility that bovine bacilli may in special cases be secondary invaders—a possibility brought forward by the discovery of both types of bacilli in the same subject.¹

In the following pages we are simply concerned in registering the results of studies made upon various cultures of tubercle bacilli which have come into our hands since 1904. Of these, Nos. XVII., XVIII., and XIX. were isolated by Dr. James Homer Wright and kindly presented to us. No. XXI. was isolated by Dr. John J. Mackenzie and sent to us for a more detailed examination. The others were isolated by one of us by passage through guinea-pigs

* Received for publication May 20, 1907.

BRIEF HISTORY OF THE CASES FROM WHICH THE CULTURES
WERE ISOLATED, TOGETHER WITH THE BACTERIO-
LOGICAL DIAGNOSIS.

Human No. XVII. — Isolated 1904 by Dr. Wright from tonsils, removed by Dr. J. L. Goodale. Growing on glycerine agar when received. The following data were kindly furnished by Dr. Goodale:

"Boy, five years of age, seen May 18, 1904, for cervical adenitis on the right side of several months' duration. Fairly well developed and nourished; color, fair; cervical glands on right enlarged to size of lima bean. Tonsils apparently normal; moderate adenoid. Adenoid and both tonsils were removed. The right tonsil on incision showed macroscopically considerable cheesy material in the center. The family physician reported on September fourteenth of the same year that the throat was clear and had given the child no trouble. The glands were slightly smaller. The general condition of the child remained unchanged."

Bacteriological diagnosis: Bovine type of tubercle bacillus.

Human No. XVIII. — We are indebted to Dr. J. L. Goodale for the following history:

"Boy, five years old. Seen April 23, 1904, with history of cervical adenitis since March first. General condition good. Both tonsils moderately enlarged, adherent to pillars. Surface smooth showing no evidence, clinically, of tubercular infiltration. Both tonsils and adenoid were removed and sent to Dr. Wright June 9, 1904. Animal inoculated with material from right tonsil shows tuberculosis of lymph nodes, spleen, and liver. Other pigs inoculated with left tonsil and adenoid show no tuberculosis.

"Boy lived in excellent environment as regards food, light and air."

In April, 1906, Dr. James S. Stone sent from the same patient scrapings from an abscess in the neck, of glandular origin. One guinea-pig inoculated with all of this material was killed ten months after inoculation. No lesions were found.

In December, 1906, there was sent by Dr. Stone from the same case some caseous material from a cervical abscess. This was injected entirely into the subcutis and abdomen of two guinea-pigs. These were chloroformed after two months, but no trace of tubercular lesions found.

Bacteriological diagnosis: Bovine type of tubercle bacillus.

Human No. XIX. — The following history of this case was kindly furnished by Dr. Goodale:

" Girl, two years old, seen in consultation Feb. 23, 1904. For three weeks fever every evening, 100.5 to 102° F., accompanied by gradual enlargement of glands under left jaw. During previous autumn she had milk for several weeks from a cow said to be afflicted with garget, which was later killed by the State Board.

" Examination: Fairly well developed and nourished; pale; several cervical glands on left enlarged to size of robin's egg, others to size of pea. Left tonsil shows moderate enlargement with marked dilatation of the crypts, the tonsillar tissue between the crypts being irregularly mammillated and prominent here and there in the form of digitations. There is no ulceration of the surface, which is covered by a glistening, translucent mucous membrane. The right tonsil is smaller and apparently normal.

" Clinical diagnosis: Tuberculosis of left tonsil and cervical glands. Both tonsils and considerable adenoid tissue removed and sent to Dr. Wright. After operation, temperature continued to run irregularly above normal, and the cervical glands were removed in March by Dr. Dane "

Through the kindness of Dr. Dane we were informed that the child was doing well in April, 1907. In the spring of 1906 the patient had an evening temperature of 100°-102°, and one gland enlarged, which seemed to vary in size. There has been no evening rise in temperature since.

Bacteriological diagnosis: Bovine type of tubercle bacillus.

Human No. XX.—This bacillus was isolated in 1905 from the urine of a case of genito-urinary tuberculosis.

The patient, a man thirty years of age, in intellectual pursuits, passed urine tinged with blood, which was sent to one of us for diagnosis. As tuberculosis was suspected, the sample of urine, containing much blood and pus, was centrifugalized and the deposit injected into guinea-pigs which became tuberculous in due time and from which the culture was isolated.

Two years later (April, 1907) we learn that the patient is doing well in a Colorado sanatorium.

Bacteriological diagnosis: Human type of tubercle bacillus.

Human No. XXI.—For the autopsy notes of this case and the culture we are indebted to Dr. John J. Mackenzie of Montreal, Canada.

Patient M. S. Male, aged twenty-one. Both lungs show extensive miliary tuberculosis; miliary tubercles small, grayish. Nowhere in either lung was there a trace of older foci or of scars. Larynx and trachea free

from ulceration. A few miliary tubercles at the bifurcation of the bronchi. Bronchial mucosa congested, no ulceration. Bronchial glands swollen, congested and show scattered tubercles, but in only one small gland near the hilus of the left lung is there any extensive caseation.

Liver, spleen, and kidneys all show extensive miliary tuberculosis.

The intestines show a few small ulcers, the largest about a centimeter in diameter. Most of them quite small with margins thickened and raised; an occasional tubercle in the serous coat.

Mesenteric glands are very extensively involved. All remarkably caseous, and those at the root of the mesentery large and matted together. The thoracic duct is normal in appearance at its lower part; at the junction of its middle and upper third there is ulceration and the wall is caseous and attached to a small caseous gland.

Dr. Mackenzie kindly sent us a culture on glycerine agar, the second transfer obtained through a guinea-pig from the mesenteric glands.

Bacteriological diagnosis: Human type of tubercle bacillus.

Human No. XXII. — This case is that of a boy three years old, who died at the Children's Hospital in Boston. The autopsy was performed by Dr. Charles W. Duval, who very kindly abstracted the clinical history of the case and prepared slides of the central nervous system as well as of many other organs of the body in which tubercles might have been anticipated.

Of the history of the case only the following bear directly upon our investigations:

Father and mother well. No consumption in family. Child breast-fed for fifteen months; not sick before.

Present illness: Became dull, sleepy, September 4th. No appetite. Became constipated, bowels moved only by irrigation. Child remained in this condition staying up around house till six days ago, when he went to bed. Vomited that night. Five days ago became worse, complained of pain in abdomen. Became restless, throwing head back on pillow. Head has never been retracted. Has been getting feverish, especially at night. Three day ago vomited again. Does not complain of headache, but of pain in abdomen. Three days ago eyes became crossed and have remained so ever since. Cries a good deal, especially yesterday, and for past five days has been cross and irritable. Has not slept much. Urine red. No nose bleed. Conscious all the time. Has been in bed last five days.

Physical examination: Fairly well developed and nourished. Looks sick. Dull mentally, but conscious. Cannot speak plainly but makes wants known. Is irritable and cries out occasionally. Pupils equal and

regular, dilated; convergent strabismus. Do not react to light. Sees very well and takes notice. No discharge from ears, eyes, or nose. Lips red and cracked. Teeth covered with sordes. Tongue coated. Tonsils slightly reddened and prominent.

September 18. Lumbar puncture in third space. Six drams of turbid fluid with fibrin flakes removed under considerable pressure. Fluid contains many leucocytes, of which eighty-three per cent are mononuclear and seventeen per cent polymorphonuclear. Child showed no change after removing fluid. White blood cells 21,400.

September 22. Convulsive twitchings; unconscious; spasm of neck muscles; no Kernig; knee jerks feeble; no Babinski; no control over sphincters. Child grew progressively worse and death occurred September 29.

Temperature on admission 102°. Pulse 110, highest point during illness 140. Respiration 30.

Autopsy fifteen hours post-mortem: Peritoneum smooth and glistening. Appendix eight centimeters in length with complete mesentery. Organ directed inward and downward. Diaphragm, left, fifth space, right, fourth space. Mesenteric lymph nodes greatly enlarged. Many are the size of marbles and very firm in consistence. On section caseous. . . .

Left adrenal contains three pin-head nodules beneath the capsule but not attached to same. Right adrenal contains four similar nodules. On section these nodules are quite firm and of a yellowish white color. Smear preparations show bacilli having the morphology and staining reaction of *B. tuberculosis*. . . .

Brain: Weight not taken. At the junction of the temporo-sphenoidal and frontal lobes in the region of the island of Reil there are a number of small flattened nodules the size of a pin head and smaller. These on section appear fatty. There is also in this region situated on the inferior surface of the temporo-sphenoidal lobe a cyst of the pia-arachnoid 2 x 1 centimeter containing clear yellow fluid which causes a slight depression of the underlying cerebral tissue. In general the convolutions are flattened. Sulci shallow, but the whole cerebral mass bulges, making the membranes appear tense. Considerable subpial edema. Cortex fairly soft in consistence. Lateral ventricles contain considerable clear fluid. Midbrain, pons, medulla, and basal ganglia negative. . . .

Careful search of the meninges fails to reveal miliary tubercles aside from those described.

Dura of spinal cord injected. On opening, the subdural space contains considerable sero-fibrinous exudate which is most marked along the cervical enlargement. The cord appears normal.

Anatomical diagnoses: Tubercular mesenteric lymph nodes; tubercular nodes in the adrenals; cyst of pia arachnoid; tubercular meningitis (cerebral and spinal).

Bacteriological diagnosis: Bovine type of tubercle bacillus.

In addition to these cultures there were examined, partly as controls, the bacilli from two cases of pulmonary tuberculosis in man.

Human No. XXIII. — April, 1906. Case of carcinoma of the breast removed four years before death. At the autopsy metastases were found in lungs, liver, and gall-bladder, adrenal, peritoneum and mesenteric lymph nodes. In addition there were multiple tubercular cavities in the lungs and areas of tubercular pneumonia (Autopsy, 1906, No. 71 of Boston City Hospital, through Dr. Mallory).

The culture was isolated from guinea-pigs inoculated with suspension of lung tissue, and found to belong to the human type of the tubercle bacillus.

Human XXIV. — Autopsy, 1906, No. 86 of Boston City Hospital. For the following brief abstract I am indebted to Dr. Mallory:

White, male, age sixty-one years: poorly nourished. Short of breath for three months; loss of weight; no cough. Alternating diarrhea and constipation.

Clinical diagnoses: Pulmonary tuberculosis; tuberculous peritonitis and enteritis

Anatomical diagnoses: General peritonitis (streptococcus pyog.); tuberculous ulcers of intestines with rupture; tuberculosis of lungs with cavity formation; caseous pneumonia; chronic mitral endocarditis; cysts of kidney; renal calculus.

Lungs: Upper right lobe contains numerous solidified patches which on section show as hard, yellow, firm areas; and in part as softened purulent areas. A few whitish areas in the lower lobe. In left upper lobe cavity at apex 8 x 9 centimeters, full of yellowish-gray, soft material. The rest of the lobe is hard and filled with small nodules. Lower lobe contains a few hard masses, largest 6 x 3 x 2 centimeters. On section they appear gray and dense, showing indistinctly lobular markings.

Gastro-intestinal tract: There are fifty-one well defined ulcers in the small intestine and thirty-six in the large intestine. These ulcers are from 1 x 1 centimeter to 1.5 x 3 centimeters in diameter. They are usually ovoid in form and lie transverse to long diameter of intestine. They are punched out in character with indurated edges. On the peritoneal surface of some of these ulcers are a few scattered white nodules. Perforation lies thirty centimeters from duodenum; opening two centimeters in diameter.

This culture was obtained from guinea-pigs inoculated with suspensions of lung tissue and found to belong to the human type of tubercle bacillus.

To complete the list it should be mentioned that a fresh culture of bovine and one of swine bacilli (Bovine IX. and Swine II.) were isolated for controls. The growth of the swine culture was, however, so feeble that the reaction curve in glycerine bouillon could not be obtained before completing this manuscript.

MORPHOLOGY AND BIOLOGY OF CULTURES STUDIED. —

The methods pursued in studying these cultures comparatively do not differ from those described in certain former articles,² excepting in regard to the culture media employed. The difficulty of obtaining dog's serum, with which one of us has had such uniform success, led to the use of egg-media followed by glycerine agar. The serum of calves fed on milk was also tried, but without much success. The blood was drawn aseptically from the jugular vein and set without repeated heatings, as is customary when blood is not obtained free from contamination. The success which others, notably Koch and his followers, have had with beef serum may perhaps be due to the repeated heatings required to sterilize the serum.

As a result of this change in the culture media, the study of the morphology of the cultures became unsatisfactory owing to the presence of involution forms on the glycerine agar.

As noted heretofore, the multiplication of the human type of bacillus on the culture media was much richer from the start than that of the bovine type. Human XXII., a bovine type, grew very feebly for a number of transfers, and was finally lost before its growth was sufficiently vigorous to permit further study in glycerine bouillon. With the human type (Nos. XX., XXI., XXIII., and XXIV.) there was no difficulty in obtaining prompt vigorous growth on the media employed, even in the first transfers. The reaction curve of the various cultures in glycerine bouillon³ agreed with the

animal tests, enabling us to make, as heretofore, a clear and sharp distinction between the bacilli of bovine and of human origin. It will be seen from the parallel cultures in glycerine bouillon in the following table (Table I.) that the culture fluid of the human type tends at first towards an alkaline, then towards a more acid condition, while the bovine type tends toward the alkaline reaction, and the culture fluid either remains alkaline or else becomes feebly acid, according to the bouillon used.

TABLE I.

Composition of Bouillon.	Designation of Culture.	Date of Inoculation.	Per cent Glycerine.	Reaction of Bouillon in per cent of a Normal Solution after: [(-) = acid; (+) = alkaline.]									
1% peptone; 1% salt; 1% and 3% glycerine. Reaction = -1.0%.	Human XII.	Nov. 9, 1905.	3	33 days = - 1.15	50 days = - 1.7	72 days = - 3.7	93 days = - 3.85	109 days = - 3.65					
	Human XVII.	Nov. 9, 1905.	1	38 days = - 0.3	129 days = + 0.6	150 days = + 0.6							
	" "	" "	3	25 " = - 0.3	47 " = + 0.7	68 " = + 0.85	88 days = + 0.35	108 days = + 0.5					
	Human XVIII.	Nov. 9, 1905.	1	30 days = - 0.4	47 days = + 0.15	68 days = + 0.45	88 days = + 0.5	108 days = + 0.3					
	" "	Dec. 28, 1905.	3	21 " = - 0.20	41 " = + 0.8	61 " = + 0.45	82 " = + 0.5	103 " = + 0.6					
	Human XIX.	Nov. 9, 1905.	1	37 days = - 0.15	50 days = + 0.35	72 days = + 0.85	93 days = + 0.7	109 days = + 0.3					
	" "	" "	3	21 " = - 0.2	45 " = + 0.8	61 " = + 0.8	80 " = + 0.45	101 " = + 0.4					
	Human XX.	Nov. 9, 1905.	3	33 days = - 0.15	50 days = - 0.85	72 days = - 0.8	93 days = - 1.6	115 days = - 2.6					
	Human XXI.	Nov. 9, 1905.	3	45 days = + 0.35	61 days = + 0.2	80 days = + 0.1	101 days = - 0.35	121 days = - 0.0					
	Bovine VIII.	Nov. 9, 1905.	1	21 days = - 0.25	45 days = + 1.05	61 days = + 1.0	80 days = + 0.75	101 days = + 0.8					
	" "	Nov. 29, 1905.	3	13 " = - 0.0	33 " = + 0.5	55 " = + 0.0	76 " = + 0.4	98 " = - 0.1					

TABLE I. — *Continued.*

Composition of Bouillon.	Description of Culture.	Date of Inoculation.	Pet. Count.	Reaction of Bouillon in per cent of a Normal Solution after: [(-) acid; (+) alkaline.]									
1% peptone; 3% salt; 3 and 5% glycerine. Reaction — 1.0%.	Human XII.	Dec. 31, 1905.	3	27 days = - 2.3	48 days = - 3.85	71 days = - 3.85	92 days = - 4.05	114 days = - 3.9					
	" "	" "	5	27 " = - 2.15	48 " = - 3.7	71 " = - 4.0	92 " = - 4.15	114 " = - 4.2					
	Human XVII.	Dec. 31, 1905.	3	18 days = + 0.15	38 days = + 0.2	58 days = + 0.0	70 days = + 0.0	100 days = + 0.5					
	" "	" "	5	18 " = - 0.55	38 " = + 0.3	58 " = + 0.0	70 " = + 0.2	100 " = + 0.0					
	Human XVIII.	Dec. 31, 1905.	3	38 days = + 0.5	58 days = + 0.15	70 days = + 0.35	100 days = + 0.3	120 days = + 0.0					
	" "	" "	5	14 " = + 0.3	67 " = + 0.2	88 " = + 0.4	111 " = - 0.1	120 " = - 0.0					
	Human XIX.	Dec. 31, 1905.	3	18 days = + 0.2	38 days = + 0.2	58 days = + 0.0	70 and 100 days = 0.0	120 days = 0.0					
	" "	" "	5	38 " = + 0.25	70 " = + 0.3	100 " = + 0.0	120 " = 0.0	120 " = 0.0					
	Human XX.	Dec. 31, 1905.	3	27 days = - 0.75	48 days = - 2.8	71 days = - 4.1	92 days = - 3.25	114 days = - 3.0					
	" "	" "	5	27 " = - 0.55	48 " = - 1.9	71 " = - 2.7	92 " = - 3.15	114 " = - 3.45					
	Human XXI.	Dec. 31, 1905.	3	27 days = - 0.2	48 days = - 1.05	71 days = - 1.5	92 days = - 1.7	114 days = - 1.45					
	" "	" "	5	38 " = - 0.6	58 " = - 0.55	79 " = - 0.8	100 " = - 1.7	114 " = - 1.15					
	Bovine VIII.	Dec. 31, 1905.	3	18 days = + 0.2	38 days = - 0.3	58 days = - 0.3	70 days = - 0.2	100 days = - 0.25					
	" "	" "	5	18 " = + 0.2	38 " = - 0.1	58 " = - 0.0	70 " = - 0.2	100 " = - 0.2					

Human XXIII. " "	July 31, 1906.	3	52 days = - 0.8	75 days = - 1.0	103 days = - 0.85	124 days = - 1.05
	July 13, "	5	53 " = - 0.45	74 " = - 1.4	93 " = - 1.75	104 " = - 1.9
Human XXIV. " "	July 13, 1906.	3	34 days = - 0.5	55 days = - 1.3	79 days = - 2.7	102 days = - 3.0
	" "	5	34 " = - 0.45	55 " = - 1.3	79 " = - 2.4	102 " = - 2.8
Bovine IX. " "	Nov. 12, 1906.	3	39 days = - 0.7	67 days = - 0.0?	89 days = - 0.55	121 days = - 0.45
	" "	5	63 " = - 1.1	85 " = - 0.0?	107 " = - 0.4	141 " = - 0.4

14 ptentone;
1/2 salt;
2 and 54
glycerine.
Reaction =
-1.85.

This table illustrates and extends the results reported by one of us in former papers. These are:

1. It is always desirable to include in the tests older cultures which have been previously tried, since different lots of bouillon are not always acted upon alike by the same culture.

2. The human type of the tubercle bacillus may be separated into two groups, one producing twice as much acid as the other. Thus, human Nos. XX. and XXIV. produce, roughly speaking, twice as much acid as Nos. XXI. and XXIII. Human No. XII., an older culture described in an earlier paper, and here included as a control, has always produced a maximum amount of acid.

Attention is here called to a slight aberrant reaction of the fresh bovine culture IX., due probably to the age of the bouillon used, as shown by the dates in the table. Owing to the relatively slow growth of this culture, other tests could not be prepared in time.

As tubercle cultures are usually dead after three months, it is not desirable to continue the reaction tests after that time.*

TESTS OF PATHOGENIC POWER UPON ANIMALS. — The cultures herein described were tested upon guinea-pigs and rabbits. Several were also inoculated into calves.

The different effects upon guinea-pigs of the bovine and the human type are not sufficiently pronounced to justify reproduction of the tests even in tabular form. The rabbit, however, serves a most useful purpose in that this animal seems to be specifically susceptible to the bovine type.⁴ In the following table (Table II.) the distinctions made by means of glycerine bouillon cultures are upheld and confirmed by the tests upon rabbits. We need simply call attention to the control tests made with an older bovine bacillus (VIII.) under cultivation for more than six years, a bovine type isolated from a child (human XI.)⁵ under

* In a painstaking study of this subject, O. Bang, following the method of one of us, has confirmed our results and has shown that avian bacilli are to be grouped with the bovine type as regards their reaction curve. *Centralbl. f. Bacteriologie. Erste Abth., Originale*, 1900, xiii, 34.

cultivation for three and one-half years, and the two fresh cultures from cattle and swine to impress the reader with the specific virulence of the bovine type for rabbits as contrasted with that of relatively fresh cultures (human XX., XXI., XXIII., and XXIV.) of the human type.

The culture medium on which the material to be injected was grown, consisted of a five per cent glycerine agar, excepting where serum is mentioned in the table. The dose injected was as heretofore a given quantity of a suspension of bacilli in salt solution equivalent in density to a twenty-four-hour bouillon culture of typhoid bacilli. The standard dose used is .5 cubic centimeter. The injection was in all cases into an ear-vein.

TABLE II.

The length of culture.	Total Age of culture.	Transfer.	Age of Culture Used.	Dose in cc.	No. of Mice.	Date of Inoculation.	Initial Wt. (grams).	Final Wt. (grams).	Result.	Remarks.
XVII..... 1 year.		?	23 days.	.5	212	Oct. 6, '05.	2850	1902	Dies in 19 days.	Dense eruption of tubercles in lungs.
XVIII..... "		?	" "	.5	210	" "	2550	1900	Chlorof. in 26 days.	" " " "
XIX..... "		?	" "	.5	209	" "	2450	1660	" " 29 "	" " " "
" 1 1/2 years.		?	10 days (serum).	.5	211	Mar. 3, '06.	2270	1555	Dies in 34 days.	" " " "
XX..... 6 months.		7th	24 "	.5	206	Oct. 6, '05.	1820	1995	Chlorof. in 2 mos. and 23 days.	Some foci in lungs and kidneys.
XXI..... 7 1/2 "		5th	15 " (serum).	.5	237	Feb. 9, '06.	2190	2110	Chlorof. in 3 mos.	Small area of red (collapsed) lung tissue in apex of one lung. Some small foci in kidneys.
XXII..... 2 months 5 days.		3d	11 " (serum).	.5	240	Mar. 3, '06.	2090	1750	Dies in 30 days.	Many tubercles in lungs, liver, spleen, and kidneys.
" "		"	" " "	.5	241	" " "	2030	1477	" " 37 "	Many tubercles in lungs, liver, and kidneys.
XXIII..... 8 1/2 months.		10th	15 "	.5	246	Feb. 27, '07.	2700	2150	Chlorof. in 3 mos.	A few tubercles in lungs and kidneys.
XXIV..... 8 1/2 "		"	12 "	.5	244	" " "	3070	2750	" " "	A moderate number of tubercles in lungs and kidneys.
Bovine VIII. ... 6 years 3 months.		81st	23 "	.5	211	Oct. 6, '05.	2630	1890	" " 24 days.	Abundant eruption of tubercles in lungs.
Human XI..... 3 years 7 "		43d	23 "	.5	207	" " "	2800	2190	Dies in 34 days.	Dense eruption of tubercles in lungs and kidneys.
Bovine IX..... 10 months.		10th	12 "	.5	242	Feb. 27, '07.	3470	2740	" " 22 "	Dense eruption of tubercles in lungs.
Swine I..... 9 "		8th	43 "	.25	245	" " "	3150	2400	Chlorof. in 44 days.	Dense eruption of tubercles in lungs, spleen, and kidneys.

The two human cultures (Nos. XXIII. and XXIV.) were also injected into the jugular vein of six calves in doses of one to five centigrams without producing more than a transient rise in temperature lasting three to eight days, whereas one centigram of culture bovine I. (over twelve and one-half years under cultivation) was fatal to a large, vigorous calf in five weeks, and one centigram of culture bovine IX. was fatal to another calf in eighteen days. These tests upon calves were made as part of another experiment, and are merely mentioned here to emphasize the different behavior of the human and the bovine type in cattle.

SUMMARY.

Among the foregoing selected set of cultures, of which three were isolated from tonsils, two from mesenteric lymph nodes, one from a case of genito-urinary tuberculosis, and two from the pulmonary form of the disease, we find the three tonsil cultures and one of the two cultures from the mesenteric nodes responding to the test for the bovine type. The rest belong to the strictly human type. Of the seven cultures isolated by one of us thus far from as many cases of presumably primary intestinal infection in man, two are thus of the bovine type.

A summary and critical analysis of all the cases of bovine tuberculosis thus far demonstrated to have been found in man will be given in a subsequent paper. In the meantime, the work of isolating and studying the bacilli of primary disease of the cervical and mesenteric lymph nodes is being continued by Dr. P. A. Lewis in this laboratory. We hope that others may be induced to utilize similar material which may fall into their hands, since the material appears to be neither abundant nor easily procured, and the work of isolation and identification cannot be hurried.

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CALCIUM METABOLISM IN A CASE OF MYOSITIS OSSIFICANS.*

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The results of investigations on calcium metabolism have been more unsatisfactory than those of any other mineral, with the exception of iron; the reason for this is that in earlier experiments only the lime of the urine was determined, that being regarded as a measure of its absorption, while the feces calcium was considered the portion unabsorbed together with a minute portion eliminated by the intestine, but which collectively always bore a definite ratio to the urine lime. No better criticism of this view has been offered than that by V. Noorden¹ more than ten years ago, which is, in substance, as follows: "We cannot fail to regard this view (the constant relation of feces lime to urine lime) as having only the worth of an hypothesis; and therefore all conclusions based upon the calcium oxide estimation in the urine alone with reference to its absorption, retention, and loss are only hypotheses." It is only in recent years that more accurate estimations of lime have given such results any value. In view of these facts it would be well, I think, to give briefly the latest results in regard to the calcium metabolism of normal individuals which is taken from Albu und Neuberg.²

This element is taken into the food either in its organic form (milk, yolk of egg, and cereals) or as an inorganic salt (carbonate, sulphate, and phosphate). In the latter form it is found in ordinary drinking water or in mineral waters. Both forms are absorbable, though perhaps not with equal readiness. The degree of absorption depends on what other salts are taken with this; for instance, sodium chloride increases its absorption, while alkalis retard it. As regards the amount of lime required daily in our food, there is much

* Received for publication May 20, 1907.

confusion. Rensval¹ was able to maintain a calcium equilibrium with only .688-.860 gram of calcium daily in his food, but this must be a minimum, and it is better to regard 1-1.5 grams calcium oxide as the daily need of every healthy adult. Any amount less than this must be regarded as insufficient.

The enormous variations in the relations of the lime in the urine to that in the feces, as well as that in the urine to the total absorbed and eliminated, must be due to the influence of the amount in food, as well as its character, upon its metabolism. Five to ten per cent of the calcium which is taken in the food is eliminated in the urine, all the rest, whether unabsorbed or absorbed and then eliminated by the intestine, is found in the feces. The amount of calcium oxide in the urine, which of course depends very largely on the character of the food, varies from .15 to .5 gram. The quantitative relation of urine lime to feces lime undergoes great variations in health and especially so in disease; in healthy adults it was found by Rensval¹ to range from 25:75 to 64:36 with an average of 44.5 to 54.5. A portion of the feces lime has been found to come from the intestinal secretions, and it has been demonstrated by Voit⁴ that in dogs lime may be absorbed by the small intestine and eliminated by the large.

We have but a limited control over the elimination of calcium by the kidneys, still something can be accomplished; for instance, Van Noorden was able to increase the urine lime by increased ingestion of water, and Hammarsten¹ increased the same factor by the use of hydrochloric acid. Rumpf² by means of lactic acid and sodium lactate, was able to increase the elimination of calcium in man fifty per cent. This increased elimination was also shown by G. Hoppe-Seyler³ to be produced by bodily rest. Food, poor in lime salts, was also shown to produce the same effect on man, while in the fasters, Cotti and Breithaupt, this fact was still further substantiated. Most of the published results on calcium metabolism in disease are of very little value because, as stated above, the examination of the feces was neglected as well as the food ingested.

Goldthwaite, Painter, Osgood and McCrudden,⁸ however, in a very carefully conducted series of investigations in the metabolism of calcium in osteomalacia, in which previous errors were avoided, demonstrated that in this disease there is a constant loss of lime. For instance, with the ingestion of 4.56 grams of calcium oxide there was an elimination of 3.85 grams of that substance in the urine and 1.8 gram in the feces, thus showing a deficit of 1.10 gram. In one other disease, pernicious anemia, the calcium metabolism has been carefully studied by V. Moraczewski⁹ with these precautions, viz.: determination of lime in the food ingested and in the urine and feces, in which an absolute loss of lime was found. A. Ott¹⁰ has also, in a manner corresponding fully with these conditions, established that if the patient is fully nourished, phthisis is not associated with any loss of lime; when, however, albuminous material begins to break down, the elimination exceeds the intake, but this is due to inanition and not to the disease. In this connection it must be mentioned that insufficient nourishment is invariably associated with increased elimination of calcium, so that a deficit results. This can be accounted for only by the assumption that the bones are broken down to a certain extent, thereby setting their calcium free. This assumption has been fully substantiated by Forster¹¹ in dogs, and by J. Munk¹² in human beings. In diabetes, also,¹¹ we come upon a much larger outgo than intake of lime, which may also be attributed to the loss of bone substance, since it was accompanied by a loss of phosphoric acid much larger than could be accounted for by loss of flesh alone, as shown by the nitrogen loss. As to the possible accumulation of lime in the animal body, little is known; in arteriosclerosis, of course, lime is stored in the arteries, but the metabolism of this process was not fully demonstrated until Rumpf, by methods absolutely beyond criticism, showed the deficient elimination of this substance in such diseases; however, the mere retention is only a coincidence of arteriosclerosis, for Herxheimer¹³ was able to produce a retention of sixteen grams calcium oxide in eight days, while Renvall⁸ retained

two and eight-tenths grams of the same oxide in thirty-two days; this was apparently stored in the bones as calcium carbonate. As neither of these individuals suffered any discomfort from these increased amounts of lime, it is fair to suppose that any abnormalities are not due to the increased amount of lime but the locality in which it is stored. The individual on whose metabolism of lime these estimations were based had been a victim for many years of myositis ossificans. On account of the peculiar nature of the complaint, it was surmised that the disease was associated with deposits of lime salts in the muscles, and hence might be accompanied by retention of lime. The patient was placed in the hospital, given the ordinary hospital diet consisting of meat, fruit, bread, vegetables, milk, and coffee with cream, but all was measured or weighed and an equal amount of every article set aside for analysis. He was allowed to eat as much as he chose. For analysis, all food was dried on a steam bath with the addition of alcohol until the weight remained practically constant, finally divided with an Empire meat-cutter, and two portions taken from different portions of the mass for analysis. The results given are the average of two closely corresponding analyses. The fluids taken were well mixed, weighed, and two portions weighed out for analysis. The urine was passed before breakfast on each day at the same hour, and collected from that time until the following day at the same time, again, of course, before breakfast. The feces were limited by a charcoal powder taken with each meal during the period of investigation. No laxatives were used. The calcium in the urine was determined by the method given in Hoppe-Seyler's Handbuch (page 420). The feces were dried with alcohol, and two portions taken for analysis; the average of which was used in the appended table:

	Daily.	Daily.	Daily.	Daily.	Daily.	Daily.	Daily.
First period, Nov. 28	Ca. O. in food, .580 g.	Ca. O. in fluids, .366 g.	Total intake, 1.246 g.	Ca. O. in urine, .472 g.	Ca. O. in feces, .787 g.	Total output, 1.257 g.	Balance, — .011.

Another point which attracts our attention is that an individual, choosing the amount of his food freely, selected that which contained a little over one gram of calcium. We also note the much larger amount of the lime taken which appears in the urine; instead of the usual five to ten per cent we have from 37.8 per cent to 42.7 per cent. The ratio of urine lime to feces lime varies so that no effort was made to calculate this factor. During the third period of seven days an effort was made to restrict the intake of lime as much as possible by choosing such foods as are poor in this substance. There were taken roast beef with .029 per cent calcium oxide based, as are all others, upon the weight of the substance dried at 120° C.; white bread with .046 per cent; grapes with .06 per cent; butter with .04 per cent; oranges with .57 per cent (given inadvertently); chicken with .039 per cent (my own estimation), and spring water with .0032 per cent of total volume. The urine was collected daily and all united for the total period, of which two estimations of the calcium oxide were made and the average taken. Feces were limited by charcoal, the total dried with alcohol and two estimations of this also made, and the total calculated from the average. Owing to a misapprehension on the part of the nurse, the different foods, after being weighed, were all placed in the same receptacle, so that instead of determining the water and calculating the lime, it was necessary to dry the whole mass with alcohol, weigh, cut finely with an Empire meat-cutter, mix well, and take two portions of which the calcium oxide was determined, and from the average the whole content determined. The lime of the water was determined from the estimation of its hardness, after deduction of the magnesium, and for this determination I am indebted to Dr. F. S. Hollis. Arranged in tabular form, we have the following:

Second period, Dec. 28.....	Total Ca. O. in food, 3.925 g.	Total Ca. O. in water, .090 g.	Total intake, 4.015 g.	Total Ca. O. in urine, 2.981 g.	Total Ca. O. in feces, 3.105 g.	Total output, 6.086 g.	Balance, — 2.071 g.
Daily average...	.560 g.	.012 g.	.572 g.	.425 g.	.473 g.	.899 g.	— .327 g.

Again we see that the metabolism follows the physiological law that when the calcium is very much restricted there is an elimination exceeding the ingestion; the elimination, as can be seen, is much less than on a full calcium food diet (1.257 grams; .890 gram), but still there is an impoverishment of the body in lime.

Sal. Goitein¹⁴ calls attention to the fact that with a diet poor in lime the elimination of this substance is reduced, but still not in proportion to the limited intake, so that there results the same reduction of the body lime which is noticed here. At times it was noticed by this author that more lime was eliminated by the intestine alone than was taken, while the urine lime never exceeded one to fifteen per cent of the total. In our case the urine lime nearly equals the feces lime (.425; .473 gram), while the former amounts to 74.1 per cent of the total ingested.

Renvall³ calls attention to this excessive elimination of lime in the urine, so that while peculiar it may not be abnormal, for his subject, a healthy individual of twenty-two years of age, in a period of fifteen days eliminated in the urine .548 gram daily and in the feces .328 gram, so that of the total lime 62.5 per cent was found in the urine. In my own investigations only 47.2 per cent was found in the urine. Renvall does not give the total calcium ingested, so that a comparison with this is impossible.

Since my work was completed an extremely interesting research on calcium metabolism by Thayer and Hazen¹⁵ has appeared. This investigation was made upon a typhoid fever patient who had received one hundred and twenty-five grams of calcium lactate by mouth and five grams calcium chloride by injection to check hemorrhages. Following this there was an abscess of the breast which, when dressed with iodoform gauze, began to deposit lime in the granulation tissue and in various nodules in the breast. Forty days after the calcium medication ceased, the experiments in metabolism began. It was found that on a milk and egg diet in three days there was a retention of lime amounting to 1.346 grams; that in twenty-one days on a carbohydrate free diet there was

an excess in output over intake of 3.672 grams, or .174 daily, while in the following ten days on a ward diet there was a retention of .602 gram. The marked elimination and disappearance of lime from the wound and nodules was synchronous.

These authors lay great stress upon the carbohydrate free diet, but it is very noticeable that during this period the intake of calcium was markedly diminished (1.171 grams, mixed diet, to .387 gram and .625 gram, carbohydrate free diet) and the less the intake the greater the excess of outgo over intake, so that their results approximate my own fairly closely in which a calcium poor diet was sought. Another point of similarity between their case and mine is the rapid increase of the urine lime during a carbohydrate free diet or, as I choose to call it, a calcium poor diet, in their case from 11.34 per cent to 40.4 per cent and in my case from 37.5 per cent to 47.2 per cent.

The case which was the subject of my study seems to offer a perfectly normal metabolism, which consists in a calcium equilibrium on a mixed diet of sufficient amount, a loss on an insufficient amount of a mixed diet, and a loss on an ample diet of foods poor in calcium. As grapes, oranges, and bread appeared in this diet, a lack of carbohydrates could have had nothing to do with the loss of lime in the body.

[In conclusion I wish to express my greatest appreciation of the kindness of Dr. Chas. F. Painter in placing this patient at my disposal for this investigation.]

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FAT EMBOLISM: REPORT OF A CASE AND OF EXPERIMENTS
ON ANIMALS.*

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The presence of fluid fat within the blood stream, first made a subject of observation by Magendie, has gradually come to be recognized as a serious pathological condition. While neither Magendie nor his immediate followers suspected the possible occurrence of the condition in man, they carefully recorded the symptoms following its artificial production in animals, and so paved the way for its later recognition as a pathological entity. Consequently, when Zenker published a case of pulmonary fat embolism, the study of such lesions was eagerly taken up, and in the half century since he wrote, numerous observations have been made upon the subject by clinicians and experimental pathologists.

As a result of such observations it has been established that fat embolism is not a rare condition, but that it is, on the contrary, a very frequent form of embolism. It occurs in slight amount in the organs of many patients dying of various diseases, and in cases of bone injury it often plays an important part. In practically every case of fracture of the bones it is probable that fat particles are set free, in greater or less number, to enter the blood stream and become lodged in the capillaries of the lungs or other organs. However, while fat embolism may thus become a frequent, and often a serious complication of primary conditions, it is but seldom that it can, of itself, cause death, and the literature records but few fatal cases. In view of this fact, the following case is reported:

The patient, J. H. M., male, aged 18 years, was admitted to the Boston City Hospital on the afternoon of Dec. 31, 1902. He had been

*Received for publication May 20, 1907.

struck and knocked down by a double-runner while coasting. After the accident he could not rise or walk. The physical condition at time of entrance was as follows: Patient is well developed and nourished, his general condition good. Pupils are equal and react normally. The mental condition is normal and there is no evidence of any cerebral injury. The chest and abdomen are negative. At the upper part of the lower third of the right leg is a point of tenderness, crepitus, and abnormal mobility. Here there is slight ecchymosis and a little swelling. There is a contused wound over the crest of the tibia, just below the tubercle. Temperature 101°; pulse 90. The injured leg was treated with pillow, and posterior and side splints. The fracture is easily held in good position. A corrosive dressing was applied to the wound.

January 1st. — Patient appeared normal in every respect during the day. Morning temperature 101.5°, pulse 96. During the evening of this day he complained of slight malaise. Temperature was now 104°, pulse 120. Physical examination was negative.

January 2d. — During the night the patient was not easily aroused for treatment, but when aroused the mental condition seemed normal, although he complained of feeling sleepy. At 6 A.M. when the ward was opened it was found impossible to arouse the patient. Temperature (rectal) 101.5°; pulse 122; respiration 28. There was slight rousing following supraorbital pressure. Pupils equal, medium in size, moved sluggishly. There was no paralysis, heart was negative, respiration shallow and somewhat distant. Abdomen negative. There was involuntary passage of urine, and a moderate spastic condition of all the limbs. Leucocytes, 13,400. The evening record shows: temperature 102°; pulse 120; respiration 28.

January 3d. — The condition was exactly the same, excepting that the temperature was more elevated; pulse was rapid and weak; respiration shallow, with bronchial rales. Examination of the wound showed no evidence of infection, no swelling, and but little ecchymosis. Patient comatose, his weakness progressively increasing. Nutrient enemata were not retained. In the evening the temperature was 104°; pulse 150; respiration 35.

January 4th. — The patient sank and died quietly at 5.15 A.M. without rousing from his coma or showing evidence of any local or general cause for his peculiar condition. Temperature before death was 105.5°; pulse 164; respiration 60.

An autopsy was performed four and three-fourths hours post-mortem by Drs. Hoag and Williams of the pathological staff. Following is a summary of the autopsy findings:

The body is that of a man, well developed and nourished, but with little subcutaneous fat. Many minute purplish areas one millimeter in diameter are regularly grouped in the skin upon the sides of the chest and abdomen. The tibia is fractured at about the middle of the right lower leg. Over the site of the fracture is a small round wound one centimeter in diameter. The leg is slightly swollen and discolored. Peritoneal,

pleural, and pericardial cavities are negative. The heart weighs three hundred and seventy grams. The valves are negative. Beneath the epicardium are a few vague purplish areas, one millimeter to three millimeters in diameter. Endocardium is smooth; beneath it are many poorly defined bright red areas, one millimeter to three millimeters in diameter, together with yellowish areas of the same size presenting a minute central red dot. On section, the myocardium and papillary muscles present many evenly distributed yellowish areas similar to those described beneath the endocardium. The lungs are of small size, the upper lobes crepitant, reddish gray in color. Section reveals considerable frothy exudation. The anterior portion of each lower lobe is crepitant, reddish gray; the posterior two-thirds is in each case purple and presents beneath the pleura many bright red areas one millimeter to two millimeters in diameter. These areas are sparsely scattered, grouped, or confluent. The smaller bronchioles contain a little grayish purulent material. The liver weighs sixteen hundred grams. On section, color is pale grayish brown; the lobules are visible, while there is seen also an occasional rounded grayish area one millimeter in diameter and of refraction slightly different from that of the surrounding tissue. The kidneys weigh together three hundred grams. The capsule strips readily, markings are distinct; cortex six millimeters to eight millimeters in thickness. Many opaque yellowish gray lines radiate from the pyramids. The glomeruli are visible as slightly elevated, reddish gray dots. The thymus gland is comparatively large and presents upon the cut surface many small reddish areas one millimeter in diameter. The brain shows a moderate injection of the larger vessels of the pia and slight subpial edema. Section reveals, in both the gray and the white matter, scattered pin-point dark red spots suggesting ecchymoses. The lateral ventricles contain an increased amount of fluid. The sinuses and middle ears are negative. The other organs presented no abnormality evident in gross. Bacteriological examination of the heart's blood, spleen, and kidney showed no organisms present.

Material from the autopsy was kindly placed at my disposal by Dr. F. B. Mallory, whose suggestions and criticism have further been of the greatest aid during the progress of this work.

Thin slices from the various organs were fixed in Zenker's fluid and in formalin, and sections were prepared by the freezing and by the paraffin imbedding methods. The paraffin sections were stained according to the eosin methylene blue method; the frozen sections were stained with "Sharlach R" and hematoxylin and mounted in glycerin.

Following are the histological findings:

Heart.—Sections stained by eosin methylene blue show nothing remarkable in the serosa or subserosa. The contents of the vessels throughout section vary markedly; many of them appear distended but entirely empty, while others are

deeply injected with red blood corpuscles. There are present many scattered, irregularly outlined areas of small size in which the capillaries appear either empty or deeply injected. Red blood corpuscles often lie free in the interstitial tissue of such areas, sometimes widely separating the muscle fibers. The latter show, in areas such as described, no change beyond a slight vacuolation of the cytoplasm or, in a few instances where there is most marked hemorrhage, signs of degeneration, that is, loss of transverse striation, slight or total loss of nuclear staining, vacuolation of the cytoplasm, and atypical cytoplasmic staining. Occasionally these necrotic areas are invaded by polynuclear leucocytes.

Sections stained with Sharlach R present a striking appearance. The areas above described are seen to be the result of an extensive blocking of the capillaries and smaller vessels by fat drops. The areas appear to the naked eye as bright red patches and under the microscope the capillaries in them are seen to be deeply injected, many of them are filled with drops or cylinders of fat, while the surrounding muscle fibers contain great numbers of fine and coarse fat droplets arranged in parallel columns corresponding to the individual fibrillæ. The fatty change is most marked in those fibers immediately surrounding the larger embolized vessels, and becomes slighter toward the periphery of the area. There are frequently small emboli in scattered capillaries outside such definite areas, but about these there is no fatty change.

Lung. — The vessels are deeply injected, the alveoli and smaller bronchioles are filled with a cellular exudate, largely of the polymorphonuclear type, though there is a varying admixture of red corpuscles, endothelial cells, and serum. Fibrin is present in small amounts only. Many areas occur in which groups of alveoli are closely filled with red corpuscles, while few leucocytes can be seen.

The Sharlach R stain shows great numbers of the larger and smaller vessels filled with fat, which markedly distends the capillaries of the alveolar walls. Frequently the boundary lines between whole groups of adjoining alveoli are

clearly marked out by the intravascular fat so that the section resembles an artificially injected preparation. Occasional fat masses are found free within the alveolar spaces and bronchioles; in some cases these occur as groups of fine droplets which appear to be undergoing a process of absorption by the surrounding phagocytic cells. The epithelial cells lining the bronchioles show a distinctly reddish staining reaction, while in many cases there occur definite intracellular fat particles which are usually arranged as a column or cone capping the nucleus at its outer end. The cytoplasm of many of the phagocytic cells of the alveoli and bronchioles contains numerous very fine fat droplets.

Spleen. — The capsule and trabeculæ are negative. The pulp tissue is hemorrhagic and many of the larger blood channels are deeply injected. Such injection is especially noticeable as a narrow zone surrounding the Malpighian corpuscles.

Sharlach R preparations show, in many of the injected zones described, groups of capillaries filled with fat droplets. The arterioles of the lymph nodules also frequently contain fat emboli, and emboli are found in smaller numbers scattered in the capillaries throughout the pulp tissue. Such embolized vessels frequently occur in groups. Many of the endothelial cells lining the blood channels show a slight reddish staining of their cytoplasm, while the leucocytes throughout the organ often contain fat droplets. Such fatty cells are found especially in the Malpighian corpuscles. The cellular content of the pulp is not remarkable.

Pancreas. — The pancreas shows marked fatty changes. Many of the capillaries of the parenchyma are filled with droplets or long bars of fat. The parenchymal cells, especially those in areas of vascular emboli, contain large numbers of fine and coarse intracytoplasmic fat droplets. In some cases the cell cytoplasm is almost entirely replaced by fat while the nuclei at times show vacuolation. The epithelial cells lining the excretory ducts show a slight fatty change. The islands of Langerhans appear to be especially affected by the fatty change; they show a comparatively greater

amount of embolism, and their cells possess a correspondingly greater fat content. There is no evidence of inflammation or of fat necrosis.

Liver. — There is a general injection of the vessels and sinusoids, often of marked degree. Occasionally vessels or small groups of vessels appear dilated and empty. There is very little vacuolation of the parenchymal cells. Sharlach R shows, however, in the liver cells, numerous very fine fat droplets. These are arranged in a narrow line extending longitudinally in the central portion of the cords of liver cells and are grouped in the individual cells in that portion of the cytoplasm adjoining the central bile capillary of the cell cord. Scattered through the sections are occasional small areas showing the sinusoids filled with fat; the liver cells here contain numerous fat droplets, while the unoccluded vessels are deeply injected. Isolated emboli also occur in small number. The emboli are usually found in the smaller vessels in the peripheral portions of the lobules.

Kidney. — The appearance of Sharlach R preparations is a striking one; many of the glomeruli show their capillary coils solidly injected with the red stained fat, while others contain amounts varying from this extreme down to a few isolated droplets. Very few, however, are entirely free. Most of the fat is lodged in the Malpighian tufts or in the smaller divisions of the interlobular arteries; only a slight amount appears to have gained the venous capillaries and the interlobular veins. It is noticeable that the vasa recta of the pyramids also contain but occasional emboli. While the network of capillaries in the outer cortical zone is extensively embolized, but little fat is found in the capsular vessels or in the stellate veins. No free fat can be demonstrated in the glomerular spaces or in the tubal lumina. There is a slight reddish staining reaction to be observed in the epithelial cells lining the convoluted tubules, while in the distal portions of the tubules — Henle's loop and onward — there is decided fatty change; the cytoplasm here contains many fine fat particles.

Adrenal. — There are occasional fat masses lying in the

sinusoids of the cortical substance. The parenchymal cells contain about the usual amount of fat.

Thymus gland. — A few of the capillaries of the adenoid tissue contain fat emboli. The corpuscles of Hassal are prominent; their cells, however, are largely broken down into masses of granular débris containing many fine fat particles. Within the lymph sinuses of the adenoid tissue are found many large cells with single small nuclei; their cytoplasm is thickly dotted with fine and coarse fat droplets. Many of the lymphocytes also contain fine fat particles.

Bronchial lymph nodes. — The peripheral and secondary lymph sinuses are dilated and contain many red corpuscles. There is considerable anthracosis. A moderate number of the vessels contain emboli, sometimes of considerable size. Within the cords of adenoid tissue and in the dilated sinuses are found numerous leucocytes and endothelial cells whose cytoplasm contains fat particles.

Mesenteric lymph nodes. — There is a marked hemorrhagic condition. The leucocytic cells of all types frequently show fat within their cytoplasm.

Brain. — There is marked injection of the vessels of the meninges and the cerebral tissues. Scattered throughout the sections, but occurring most often in the white matter, are small rounded areas of closely crowded red blood corpuscles. The corpuscles are in some places included within the walls of a vessel, in other places they occur as an extravasation the point of whose origin does not appear, or they surround a small vessel whose perivascular space they crowd. The ganglionic cells of such areas sometimes show degenerative changes of very slight extent. Sharlach R preparations show the capillaries of the cortex extensively filled with drops or cylinders of fat, while similar emboli occur, in slightly lesser numbers, throughout the white matter. About the embolized vessels there are frequently found zones of hemorrhage and early degenerative change. Many of the large multipolar cells of the cortex show fat within their cytoplasm; this is at times only sufficient to give a slight reddish staining reaction, but in other cases definite fat droplets may be found.

Sections of the Pons Varolii show a similar blocking of the vessels by fat masses, while the large cells of the ganglionic areas show a fat content similar to that observed in the cortex.

The lesions found in this case are, therefore, fracture of the right tibia, minute yellowish areas in the heart, and ecchymoses in several organs, including the skin. Microscopically there are emboli of fat in the smaller arteries and in the capillaries of all the organs, but most abundantly in the heart, lungs, spleen, kidneys, pancreas, and brain. In two of the organs, the heart and brain, secondary lesions (degeneration of the cytoplasm and deposition of fat droplets) have followed the blocking up of some of the vessels. The clinical symptoms and death seem, therefore, unquestionably to have been due to fat embolism following the fracture.

The literature presents an interesting succession of theories as to the origin and action of fat circulating in the blood stream. The earliest observers of fat embolism did not understand the results obtained in their experiments. Thus, according to Flournoy, Lower, as early as 1669, appears to have produced the condition in the dog by the intravenous injection of large amounts of milk. Donne, in 1846, in trying to prove experimentally the origin of the blood corpuscles from the fat globules of milk, injected large amounts of the latter fluid. This resulted in the death of many of his animals but he did not examine them post-mortem. Magendie first reported the now classical symptoms of fat embolism in dogs treated with intravenous fat injections; noted the presence of fat in the vessels of the lung and in the liver cells; and believed that the temperature rise observed in such animals depended upon this vessel blocking. He further deduced the theory that the temperature of fevers is due to a similar action of blood thickened as the result of free sweating.

Though a few observers carried on similar experiments during the next thirty-five years they added nothing, and the early observations had been almost forgotten when interest in them was revived through the use of oil injections by Virchow and others during the progress of their study of pulmonary embolism. Then the whole subject was removed from the field of purely experimental research and given a more human interest through the observation by Müller (1860) of fatty embolism of the choroidal vessels of a young man, dead of interstitial nephritis, who, before his death, had suffered from amblyopia. Next Zenker (1862) reported the first case of pulmonary fat embolism in man. The condition followed an extensive crushing injury of the thorax which had produced a rupture of the liver and of the pylorus. Zenker believed that the intravascular fat came from a passage of the fatty stomach contents directly into the ruptured hepatic veins. In the same year Wagner described a similar blocking of the pulmonary vessels in a case of pyemia, and concluded that the free intravascular fat was the cause of the metastatic abscesses found in his case. He believed that the fat lodging in the vessels set up in the surrounding tissues a localized inflammatory process. Thiernes had had a similar idea the year before in describing a "Pneumonia oleosa" which he had produced by intravenous oil injections.

With the question now well opened, many workers applied themselves to its study. Grohe and Lanceraux added little. Then Bergmann took up the work. He injected lard warmed to body temperature into the great saphenous vein of cats, using quantities as high as six grams. He noted fat embolism of the lungs, with infarctions and edema, and found emboli in the kidney and liver of animals surviving the injection for from six to twenty-four hours. He also records the presence of free fat within the uriniferous tubules, and concluded that it escaped into them through the walls of the glomerular vessels. In support of this theory he demonstrated the presence of fat in the urine during life. He believed that while fat embolism was rarely of great primary

importance, it might become an important complication of primary conditions, and concluded that death was caused in fatal cases by a paralysis (Lähmung) of the heart, associated with pulmonary edema. He maintained that the extensive blocking of the pulmonary vessels resulted in a deficient aeration of the blood, and that such impure blood was unable properly to nourish the cardiac ganglia, so that paralysis resulted from this failure of nutrition.

The problem of etiology was now made of interest by Busch. This observer, working under Von Recklinghausen, first pointed out the connection between fractures of bones and fat embolism. He studied the effects upon dogs of traumatic injuries of the bones and marrow, and by the use of cinnabar injections into the marrow cavity showed that the fat was taken up there by the ruptured veins, and to much less extent by the lymphatics. He believed that most of the fat found its way into the circulation in from a few minutes to three hours after the receipt of an injury, and concluded that death was one of the cases of the circulatory disturbances following extensive embolism of the lungs, kidneys and brain.

He agreed with Bergmann in regarding the fat embolism like importance as a primary cause of death. It must be justice to say that Wagner whose theory of the formation of fatty embolism was given a bad name by Busch, was endeavoring to go further work upon the subject. As a result of these later observations he arrived at conclusions similar to those of Busch, endorsing them in a paper published after the appearance of that of the latter.

The next development was the production of "fumes" of petroleum smoke and through the ingestion of fat into the general and peripheral circulation and also through its entrance into the subcutaneous tissues without the rupture of any blood vessels. In the latter case the process was found to be longer delayed on account of the filtering action of the lymph nodes through which the fat particles must pass before gaining entrance into the blood stream. "Fumes" believed that the fat was largely absorbed by the skin's

though some was probably emulsified and absorbed by the lymphatics. In strong dogs he found that all the fat was eliminated in from three to four weeks. Riedel and Scriba supported this work by their own experiments. Riedel, as well as Busch and Flournoy, stated that the fat could be taken up even by the intact lymphatics. Hahn, after fracturing and smashing bones, showed that emboli could be found immediately in the lungs, heart, liver, and kidneys. He believed that the fat was eliminated by the kidneys and in part by exudation through the capillary walls into the surrounding tissue, from which, as already stated by Bergmann, it is gradually absorbed. He did not succeed in producing embolism by extensive laceration and breaking up of the subcutaneous fat or of that of the mesentery, and concluded that the conditions most favorable for absorption are found in the marrow cavities of the bones.

As a result of the work thus far completed, the problem had grown in importance, while at the same time it took on more and more a definite outline and meaning. It remained for Scriba to gather together the more or less loose threads left by former observers and to give the subject logical form. While exceptions must be taken to some of his conclusions, his work was thorough, and its careful and logical method served to clear up many previously obscure points. His most important conclusion was that death occurred only after extensive embolism of the brain and cord, and that it was not dependent, to appreciable degree, upon pulmonary or cardiac derangement. While Hahn, Czerny, Flournoy, and others had called attention to the cerebral symptoms observed clinically in fatal cases, and though Bergmann had considered the possibility of a cerebral factor as a cause of death, Scriba was the first to insist upon the great significance of cerebral embolism. This led, he believed, to deficient oxygenation in the vital centers and thus determined death. From his experiments upon frogs he supported Bergmann's idea that fat excretion took place through the capillary walls of the glomerular vessels and not through the epithelial cells of the tubules; he further pointed out the peculiar periodical

appearance and disappearance of free fat in the urine of fracture cases. He insisted on its demonstration as a valuable aid in diagnosis. Another diagnostic point which he emphasized is the appearance, in all cases, of a fall in temperature; this is especially marked in those followed by death. He showed that, in general, a fatal result followed only after an amount of fat equal to three times that contained in the animal's femur had entered the circulation; the exact amount depends to some extent upon the distance of its point of entry from the heart and upon variations in the *vis a tergo*.

Since Scriba's work appeared, little has been added to our knowledge as to the ultimate action of fat embolism, though the frequency of its occurrence, in minor degree at least, in many varied conditions has been shown to be much greater than was at first suspected. It is now recognized that fat embolism in varying amount may appear in any of the various disturbances following the action of trauma or of inflammatory conditions upon tissues rich in fat. Flournoy, among two hundred and fifty bodies examined at the Pathological Institute at Strasburg, found fat emboli in twenty-six; only five of these cases were traumatic bone lesions. Of the remaining cases there was one of acute osteomyelitis; two of operation followed by acute inflammation in fatty tissues; five of inflammation of soft parts and bone together; one of multiple contusions of the subcutaneous adipose tissues in an insane woman, while nine cases showed neither trauma, inflammation, nor other condition usually held responsible for fat embolism. Three cases are excluded because of the lack of sufficient data. Carrara, out of one hundred and two cases examined, reports emboli in twenty-eight. Thus, of twenty-seven cases of disease of the lung and kidney, it was found in twenty-two per cent; in thirteen cases of burns and scalds, in forty-four per cent; among seventeen cases of fracture, in seventy-six per cent; in five cases of phosphorus poisoning, in twenty per cent. Moullin, among fourteen cases taken at random, found fat emboli in twelve. In only one of these had the condition been suspected during life. Riedel demonstrated emboli in two cases of injury to the soft

parts and in three cases of osteomyelitis. Jolly reports three cases after simple mechanical rupture of the fat cells of the subcutaneous adipose tissue. Saundby and Barling, in an examination of ten cases of wounds and injuries, found pulmonary fat emboli in eight; in all the cases death was due, however, to other causes.

Such findings indicate the comparative frequency with which free fluid fat gains entrance to the blood stream, though in many such cases it is evident that the process is so slight as to have no appreciable significance. The recorded cases in which fat has been demonstrated in quantities sufficient to cause death, and in which it constitutes the only pathological condition found which could account for death, are as rare as this slight embolism is common. Scriba, from a total of eighty-six cases collected by him, could accept only eight from the entire number as fully proved; two others were regarded as probably genuine, but were thrown out because of insufficient data. Meeh, in 1892, reports two cases from Brun's clinic, and from a review of the literature brings the total of recorded cases to one hundred and thirteen. Of the twenty-five added to Scriba's list, only five to seven can be accepted as genuine, so that the total of well authenticated cases is set down as thirteen to fifteen. Jentzsch, in 1898, collects five further cases and reports three more which had occurred among two thousand fracture cases at Bramann's clinic. Since 1898 there may be added the single cases reported by Pomatti, Payr, Seegers, and Joachim.* A further case reported by Engel is of doubtful value.

A rather careful review of the English and American literature has revealed a disappointing paucity of material upon this subject; the cases reported are usually so imperfectly presented as to be of little value. From England, apparently authentic cases have been reported, however, by Prichard, Coats, Skirving, and Hamilton. Skirving's case

* Since this paper was written, two cases have been reported by K. Brenziger (Wien. Klin. Rundschau., 1906, xx, pp. 505-507). He refers to an additional case reported by Preindlsberger (Zeitschr. f. Heilk., 1902).

followed forcible extension of an ankylosed joint, while Hamilton's resulted from the rupture of a very fatty liver. It must be said, however, that all of these cases leave much to be desired in the matter of detail. Even less satisfactory are cases reported by Southam, Saundby and Barling, Hirsch, and Ferguson. The latter's case followed free incision of a breast abscess. Only rarely have these observers reported an examination of the brain; they looked only to the lungs for the presence of emboli or reported other organs "negative."

The American literature is scanty. Bailey appears to have been the first to record a case, but it is evident that his patient died of septicemia rather than of embolism. Whitney and Fitz record cases in which they considered fat embolism as at least a contributing cause for death. Fitz also reports an undoubted case following a dislocation of the hip. The fat was set free through the laceration of the adipose tissue surrounding the hip joint. Single cases reported by Fenger and Salisbury, and by Claussen appear genuine but are insufficiently recorded. The former writers found emboli in lungs and brain. The latter reports emboli in the lungs alone, none of the other organs having been studied microscopically. Bard and Bonine and Belknap report diagnoses of the condition, but as the patient in each case recovered, there was no opportunity for autopsy confirmation. It is significant, however, that in the latter's case free fat was found in the urine on the nineteenth day. Isolated reports of other cases have appeared but only in too meager detail to warrant their acceptance. It may be noted that Brown and Starr, as well as a few English writers, chief of whom are Sanders and Hamilton, have reported cases of Diabetes mellitus with "milky blood" in which death is ascribed to fat embolism. This theory is rejected by Fitz but is accepted by Shattuck as an explanation for the diabetic coma and death. A case of diabetes with marked oligemia fortunately came under observation during the progress of this work, and the microscopic appearances noted (a fine emulsion of fat) differ so widely from those occurring in true

fat embolism as to throw strong doubt upon the existence of any possible connection between the two conditions.

As to the exact nature of the lethal action of fat emboli, there has been, as above indicated, considerable discussion. The early observers were particularly impressed by the pulmonary lesions; Bergmann, Riedel, Flournoy, Wagner, Schweniger, and others ascribed death to pulmonary edema together with cardiac disturbance. Such heart condition was believed to be due to the deficient nutrition afforded by the poorly aerated blood. But it was early noticed that emboli were easily demonstrated in other organs—thus, Cohn saw emboli in the capillaries of the brain and limbs; Bergmann noted widespread embolism, especially in animals surviving oil injections for several hours. Busch doubted the single importance of pulmonary lesions and concluded that death was due to the great circulatory disturbances following massive embolism of all the great viscera. Though Bergmann had suggested the possible importance of the cerebral emboli seen by him, Czerny was the first to call particular attention to this lesion. His contention was vigorously taken up by Scriba, who showed, as did Wiener, that death might occur in experimental animals showing no edema of the lungs. Clinical symptoms apparently cerebral in origin had been noted by Halm, Czerny, Flournoy, but these men had merely suggested their significance, or, in the case of Flournoy, had considered them of entirely secondary importance. Scriba, on the other hand, declared emphatically that the only lesion capable of producing death was embolism of the brain and cord, that fat embolism had no injurious effect upon the heart, and that if pulmonary edema ever appeared, it was so trifling in extent that it could be disregarded. This idea has been much opposed and von Zwicke, von Lympius, Meeh, and others have published typical cases showing no cerebral emboli. Seegers, it may be noted, while studying the cerebral lesions, produced fatal embolism by the injection of sterile fat into the common carotid artery; the animal showed emboli nowhere but in the brain, where they were present in both hemispheres.

These opposing views may, however, be harmonized. The important point to be considered, as recently noted by Barack, is the time between the entrance of the fat into the circulation and the occurrence of death. The study of the recorded cases, as well as the consideration of the findings in the experiments given below, would seem to indicate that we may have two modes of death. The first is found in those instances in which death occurs in a few hours; such cases present a massive embolism of the pulmonary vessels, usually accompanied by edema of greater or less extent. The clinical symptoms are those of "shock" or asphyxiation. The second appears in those cases surviving for periods of twenty-four hours or longer; the symptoms here usually appear first on the second to the fifth day. In these cases the pulmonary embolism is still great, though evidently not sufficient to cause death of itself, for experiments show that death does not occur from circulatory stasis in the lungs until fully five-sixths of the pulmonary vessel area has been shut off. But in these cases the fat has passed the lungs; has gained wide distribution throughout the body; and has produced lesions of all the vital organs. Among them the brain and cord suffer to a marked degree, and it is in such cases that clinical and pathological findings point toward lesions of the central nervous system as an important factor in the lethal action.

The case here presented is an example of this latter type. The patient survived injury for eighty-four hours, the first twenty-four of which were without clinical evidence of mishap. From about the thirtieth hour onward the patient showed, however, symptoms of cerebral disturbance; these became gradually more marked and were attended by symptoms apparently referable to lungs and heart. While the temperature was elevated, there was no sign of infection of the small skin wound near the site of fracture, and bacteriological examination after death showed no evidence of sepsis. Despite Scriba's conclusion that there is always a temperature fall in typical cases, the rising temperature noted in this case has been observed and recorded by numerous

writers in well authenticated cases. Moreover, the gross and microscopical appearances are those of widespread embolism. But while the lungs, heart, and brain show in gross evidence of vessel blocking, there is little convincing evidence of disease until the tissues are stained for fat and examined microscopically. In such preparations the heart lesion is especially striking. Although Busch, Seegers, Jentsch, and von Recklinghausen note fatty degeneration of the myocardial fibers, the condition seems not to have received the consideration it deserves; von Recklinghausen alone insisted especially upon its importance. Yet the change, as seen in the present case, is great, while the resulting disability occurs at a time when the maximum of energy is needed for dislodging the emboli blocking the capillaries throughout the body.

The extensive blocking of the kidney glomeruli lames an important portal of exit, and perhaps determines in part the pulmonary edema, as was suggested by Wiener.*

Of the other great viscera, the embolism of the spleen and liver do not appear important, while it is difficult to estimate the possible significance to be attributed to the extensive fatty change observed in the pancreas. Cerebral involvement in the present case is of marked degree; the finer terminal vessels of the gray matter are blocked in numbers by the fat, while those of the white matter do not escape. There is unmistakable evidence of early necrotic change in the tissue about such blocked vessels, as has been noted by Joachim and Pomatti. Seegers concludes that such pathological alteration of the cerebral tissue first prepares the way for the onset of extravascular hemorrhage. In these cerebral alterations there appears sufficient explanation for the drowsiness, followed by unconsciousness, spasticity, and coma observed clinically, and for the partial paralyses recorded in other cases.

In connection with the unusual persistence of the thymus gland in this patient, it is interesting to note reports by Payr,

* Scriba believed that such edema was not merely the result of the filtering of serum through the walls of engorged blood vessels, but that it was due to vasomotor disturbances produced by the cerebral lesions.

Alrent, and Colley of the presence of a status thymicus et status lymphaticus in their cases of fatal fat embolism following "brûlement forcé."

The elimination of fat has been shown to take place largely by its escape into the uriniferous tubules through the walls of the glomerular capillaries. Scriba believed that a small amount was disposed of by the leucocytes, and describes fat droplets in the cells of the splenic pulp. Michal notes a similar appearance, while Riedel and Hohlbeck described in the lungs large cells containing fat particles. Such observations are borne out by the appearances found in the present instance. The leucocytic cells of spleen, lungs, thymus, and lymph nodes are found to contain small fat droplets, often in large numbers. There is here an indication of the ingestion of fat by such cells, and it appears probable that a considerable amount may be disposed of in this way. The appearance of fat masses in the epithelial cells of some of the organs suggests the possible participation of the latter in the process of elimination.

With the object of observing, if possible, the organ or organs showing a preponderance of grave lesion following the presence in the blood of free circulating fat, experiments were carried out upon a series of rabbits. Fat in measured quantities was injected into the dorsal ear vein, observation was made of the resulting symptoms, and sections of the various organs were cut upon the freezing microtome and stained with Sharlach R and hematoxylin. Specimens were imbedded in gelatin before freezing, when necessary. The fat used was at first cotton-seed oil; a second series of animals was treated with animal fat prepared from absolutely fresh suet, and maintained as nearly as possible free of fatty acids. The difficulties here were so considerable, however, that the cotton-seed oil, which was demonstrated to be acid free, was again taken up. The action of the vegetable oil was in no way different from that of animal origin.

The results of these experiments may be shortly summarized. The cases were seen to fall into two groups, as has

been noted above; first, those resulting from the injection at one operation of a large amount of fat; and second, those developed by the injection of small quantities of oil repeated at intervals of from a few hours to three days. Rabbits I., X., and XI. received injections of from one to three and a half cubic centimeters of oil. In all, death followed in an average time of three minutes and was preceded by great dyspnea, muscular weakness, and limited convulsions. Histological examination of the organs shows great numbers of emboli in the pulmonary vessels. The heart, except in Rabbit XI., shows marked fat embolism of its vessels, and in all there is the presence of small emboli of the renal capillaries, though it is noticeable that the fat masses do not appear in the glomerular vessels. The central nervous system is in all cases free of emboli. In all these animals the heart showed faint rhythmic contractions when the thorax was opened for autopsy — in one case the animal had then been dead for ten minutes. It appeared evident that death was due here to a practical shutting off of the lungs en masse, so that the animals died of asphyxiation. Marked "air hunger" was apparent and death was sometimes preceded by spasmodic attempts at abdominal breathing. Artificial respiration in one case met with a slight response.

More interesting, as more closely approaching the condition in the human case reported, were the findings in animals treated by repeated oil injections. Such animals received total amounts varying from 1.2 cubic centimeters to 2.8 cubic centimeters of oil in injections of from .2 cubic centimeter to .8 cubic centimeter; the whole period of treatment lasted in different cases from three to seventeen days. In connection with the dosage it was observed that different animals showed marked variations in their resistance to proportionate amounts of oil, so that no arbitrary standard could be fixed for the minimum fatal dose. It was apparent, however, that such amount, for animals averaging eleven hundred grams in weight, could roughly be stated as about one cubic centimeter. In sharp contrast with this average fatal quantity is the fact that in many cases primary single

cases of 1 cubic centimeter were followed only by a slight passing dyspnea or motion, by no evidence of any discomfort.

The pathological findings in this second group are in the next case (Animal VII) may be taken as a type example. It was an animal weighing eighteen hundred and twenty-five grams and was given a total of 1.0 cubic centimeters of cottonseed oil in three injections extending over a period of five days. Its organs show with particular clearness the lesions found in human cases, particularly in all the animals of the group. While, on the other hand, they present a striking similarity to those of our human case. The heart is extremely embolized, and the blocked vessels are surrounded by many areas of extreme fatty change of the myocardial fibers. The lungs show marked embolism, though this is very clearly less in amount than is observed in animals killed by large injections; the kidney glomeruli are everywhere plugged with fat while brain and cord show frequent emboli. Those in the brain frequently surrounded by a zone of hemorrhage or early necrotic change. An interesting point also is the fact that the thymus is comparatively large and presents a microscopic appearance closely comparable to that of the human case, even as to the presence of the large fat bearing cells of its lymphoid tissue. Clinically, this case showed typical symptoms. It gradually developed a condition of apathy, muscular relaxation, and dyspnea, the pulse and respiration increased. Death occurred forty-eight hours after the last injection and was preceded by a convulsive attack lasting about ten minutes.

A review of the findings in the remaining nine animals of this group brings out several interesting points. Animals II., III., and IV. each received three injections, II. received a total of 1.5 cubic centimeters of oil during a period of eight days; III., a total of 2.0 cubic centimeters in three days; IV., a total of 2.2 cubic centimeters in nine days. In all, the third injection was fatal in a few minutes, the amount being 1.0 cubic centimeter for number II., .8 cubic centimeter for III. and IV. These animals show as a dominant

lesion great pulmonary embolism and this is apparently the chief factor in producing death. But along with the pulmonary changes go those characteristic of an embolism of longer duration. Thus II. shows a marked change of the myocardium, while all show emboli of the kidney glomeruli and of the brain and cord. A peculiar lesion observed in IV. is a fatty change of the muscle fibers of the diaphragm analogous to that above described as occurring in the heart. The fat droplets appear in beadlike chains and are found to occur as small particles strung along in regular order in the myoplasm of the individual fibrillae; the lines of cross striation separate successive globules. This animal was the first in which the diaphragm was studied; routine examination thereafter showed the presence of the lesion in five of the eight animals examined. So far as can be ascertained no previous record of this lesion has appeared, and while its significance is problematical, it is hoped that future observation may be made with the view of ascertaining the value to be placed upon it. The diaphragm of the normal rabbit has not been found to show any similar condition.

Rabbit XIII. shows lesions paralleling those of VII. (above described). It received a total of 1.4 cubic centimeters of oil given in seven injections during a period of three days. Death occurred during the night of the third day. There are found, as in VII., moderate pulmonary embolism, marked embolism of kidney glomeruli and of the central nervous system, and fatty change of the cardiac and diaphragmatic muscle fibers. The pulmonary lesion is here outweighed by the changes in the other organs.

Rabbits V. and VI. received, respectively, 2.6 cubic centimeters of oil in fourteen days, and 2.8 cubic centimeters in seventeen days. The injections were gradually diminished from an initial dose of .8 cubic centimeter to one of .2 cubic centimeter. Both animals remained free of symptoms and were finally chloroformed a few hours after the last injection. They show a widespread embolism involving lungs, heart, kidney, diaphragm, and central nervous system. While there is slight fatty change of the heart and diaphragm, with diffuse

blocking of the smaller vessels in the other organs, the changes are nowhere marked. Such changes, which differ only in degree from those found in fatal cases, indicate that the organism may resist the action of a considerable amount of fat provided such fat enters the circulation in small quantities and at comparatively long intervals. All the last mentioned animals were found to excrete fat in the urine, while the leucocytes of the lymphoid structures and of the tissues appear to be participating in the removal of the substance. In the lungs the fat is often accumulated into more or less circumscribed areas. Here it appears as though broken up into small particles, and in such areas there appear thickly clustered phagocytic cells, many of which contain large or small fat globules.

Finally, mention may be made of Rabbit IX. This animal, of the same litter as VII., and of almost exactly the same weight, showed no symptoms of disturbance after receiving exactly similar treatment. After an interval of one month it was given three injections of .2 cubic centimeter each during a period of twelve hours. Dyspnea, drowsiness, and muscular weakness developed within an hour after the last injection and death occurred during the night. Here there is a slight but widespread embolism involving heart, kidneys, liver, spleen, and central nervous system, together with an extreme blocking of the pulmonary vessels. This animal, after having eliminated almost entirely the fat given at the previous time, has been overwhelmed by a comparatively slight additional amount of oil. The case illustrates the soundness of the surgical law forbidding any avoidable manipulation of recently fractured bones, since there is always the possibility, in such procedure, of adding to the fat already in the circulation an additional amount sufficient to produce a fatal result.

CONCLUSIONS.

1. Two lesion complexes may be found in fatal cases of fat embolism. The first consists of an extreme blocking of

the pulmonary vessels, together with a less marked involvement of those of the heart. There is, at most, only a negligible blocking of the vessels of the general circulatory system. The second involves widespread embolism of the blood channels; the pulmonary lesion is here of secondary importance and is overshadowed by lesions of the heart, kidney, and central nervous system.

2. Death occurs in the first class of cases from asphyxia, and follows closely upon the trauma or disease leading to the entrance of fat into the vessels. In the second class of cases it depends upon multiple cerebral emboli, associated with embolism and fatty degeneration of the heart. In such cases death follows only after the lapse of some days (usually three to eight).

3. The dividing line between fatal and non-fatal amounts of fat is ill-defined. Individual susceptibility appears to be widely variant.

4. An amount of fat, which would be fatal if suddenly gaining entrance into the blood stream, produces no unfavorable symptoms if it enters the circulation in divided doses separated by intervals of several days. Such fat is gradually eliminated through the kidneys and by the phagocytic action of the leucocytes.

5. Fatty degeneration of the heart in fatal cases is often accompanied by similar changes in the diaphragm; the skeletal muscles remain unaffected. Such diaphragmatic lesion may in part account for the respiratory disturbance always observed.

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DESCRIPTION OF PLATES.

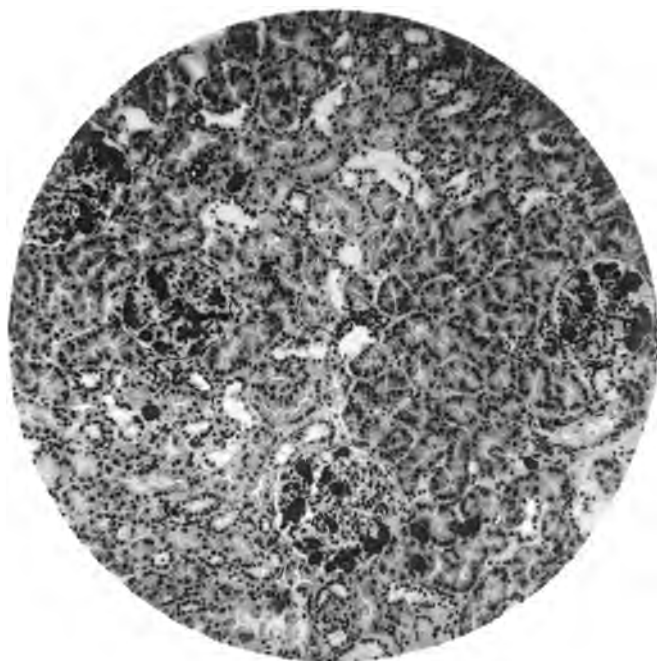
PLATE XXII.

Distribution of fat emboli in the glomeruli of the kidney. Fig. 1, low power; Fig. 2, high power.

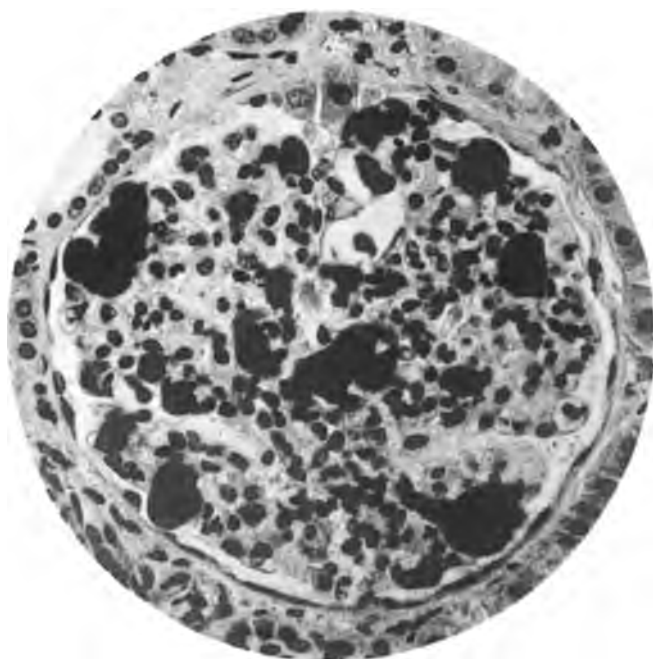
PLATE XXIII.

Heart showing fat emboli and degeneration of a group of muscle fibers. Fig. 1, low power; Fig. 2, high power.

(Photographs of the specimens were made by Dr. S. B. Wolbach, whose kindness the author wishes to take this opportunity for acknowledging.)

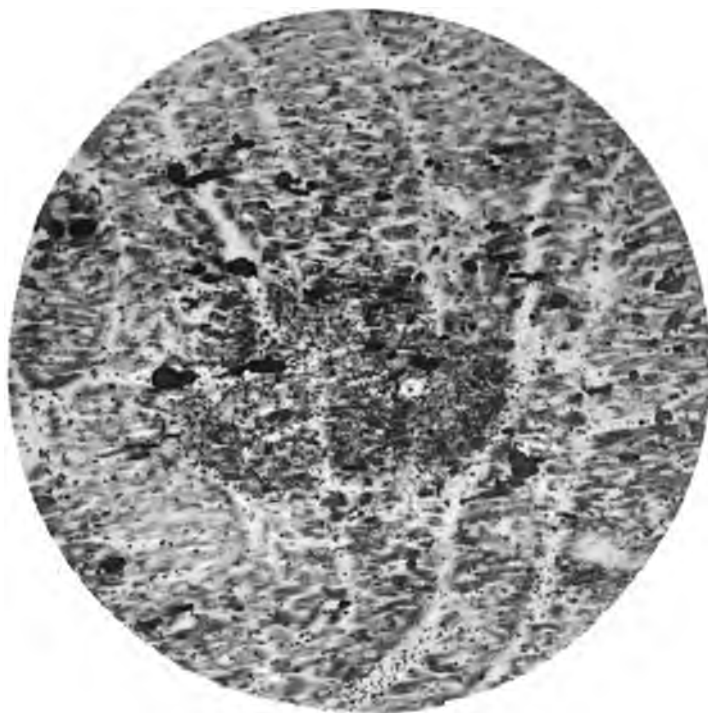


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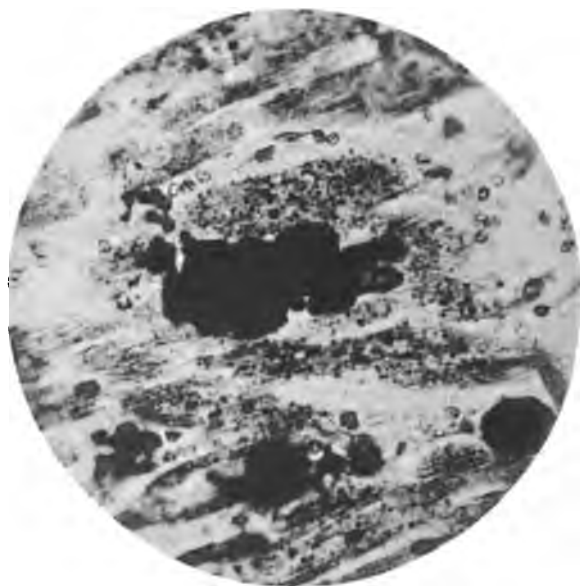


2





1



2

A CASE OF MULTIPLE PRIMARY CARCINOMA.*

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The comparative rarity of multiple primary malignant growths seems sufficient excuse for the presentation of this case particularly as there are several possibilities in regard to the nature of one of the growths.

The case is as follows:

E. V. White; age, 87. — Admitted to hospital Sept. 21, 1906. Family history: Negative. Past history: Diseases of childhood. Present history: About six or seven months ago a small yellowish spot appeared upon the right upper lip, at the same time a similar spot occurred on the back of the right wrist. These soon became ulcers. These areas were treated at various times with plasters but without any favorable results. Physical examination: There is present a sore extending from the right nasal opening to the edge of the upper lip and from there to the right angle of the mouth. This ulcer communicates with the mucous membrane by means of a fistulous opening. The submaxillary and the sublingual nodes on the right side are perceptibly enlarged. On the back of the right wrist is a small scab-covered ulcer about one centimeter in diameter.

While in the hospital there developed in the left cheek a mass that was freely movable, not being adherent either to the skin or to the mucous membrane. This began about two months before death and at the autopsy had attained a size of about 3 x 2 centimeters. At this time the left sublingual and submaxillary nodes and one of the supraclavicular as well were considerably enlarged.

At no time were there any gastric symptoms. The appetite remained good up to the day of death.

The following are the post-mortem notes and the results of the microscopic examinations:

E. V. Age, 87; female; white. — Died Dec. 16, 1906, 4 A.M. Autopsy Dec. 16, 1906, 3 P.M. Weight of organs: Heart, 355 grams; left lung, 400 grams; right lung, 610 grams; spleen, 45 grams; left kidney, 90 grams; right kidney, 90 grams; liver, 910 grams.

Pathological diagnoses: Epithelioma of face, epithelioma of wrist, epithelioma of cheek, carcinoma of stomach, carcinoma and tuberculosis of gastric nodes, carcinoma of liver, bilateral obliterative pleuritis, healed

* Presented before the American Association of Pathologists and Bacteriologists, Washington, D.C., May 7, 1907. Received for publication May 27, 1907.

tubercles of both apices, obliterative pericarditis, interstitial nephritis, atrophic spleen, calcified fibro-myoma of the uterus, atheroma of aorta and coronaries.

The body is that of a small, much emaciated adult white female. On the right upper lip from the outer angle to the ala of the nose is a rough, irregular area in which there is a small sinus communicating with the inner mucous membrane. On the right wrist is a small scab covering an ulcer about one centimeter in diameter. In the muscle of the left cheek is a freely movable tumor about two centimeters in diameter.

There is very little subcutaneous fatty tissue. The abdominal organs are free from adhesions. Appendix small and fibrous. Omentum contains very little fat.

Right pleural cavity is completely obliterated by adhesions. The left cavity is not completely obliterated, contains a small amount of serous fluid.

Heart. — Pericardial cavity completely obliterated. Heart muscle rather pale. Some atheroma of aortic leaflets and of coronaries.

Lungs. — Both lungs showed practically similar conditions. They were pale and emphysematous, contained a small amount of clear fluid in some areas. Both apices were the seat of quite large depressed scars. The left apex was collapsed and fibrous, atelectatic. Bronchi contained considerable muco-purulent exudate.

Spleen. — Was very small and dense. Capsule contained a few small whitish nodules collected in a group. Follicles were not visible. Trabeculæ quite plainly seen.

Kidneys. — Both organs very small, pale in color, quite hard. Numerous small cysts visible beneath capsule. Capsule when stripped removed portions of renal tissue. Cortex thin.

Adrenals. — Apparently normal.

Pancreas. — Apparently normal.

Stomach. — Small. The anterior wall in the region of the cardia was covered externally by many small, irregular, whitish dense nodules and flattened areas. The glands along the lesser curvature were enlarged. On opening the organ there was found an elevated necrotic area about two centimeters in diameter situated in the greater curvature just beyond the opening of the esophagus. On the anterior wall about one-third the distance from the cardia was another involved area about four centimeters in diameter. This was considerably elevated and quite necrotic in its central portion, giving it an umbilicated appearance. On the posterior wall, symmetrically situated, was a growth almost similar except that it was a trifle smaller. On the anterior wall about two centimeters beyond the above growth was another similar circular involved area as well as a somewhat diffuse infiltration. There were also corresponding lesions on the posterior wall. Esophagus was clear throughout and there were no enlarged nodes along its course.

Liver. — Small, mottled by distinct irregular yellowish and brown areas. Scattered over the surface and throughout the substance were many areas, either yellowish in color or mixed yellow and red from hemorrhage. When located on the surface these areas were slightly raised. Was very little difference in density from the liver tissue itself.

Intestines. — The large intestine showed on its external surface many diverticula about the size of a split pea. These contained fecal matter and communicated with the lumen by a small opening.

Uterus. — Was represented by a very hard mass about the size of a man's fist, at the lower portion of which was a remnant about two centimeters long of the cervix. The mass was so infiltrated by lime salts that it was necessary to use a saw to cut into it. Ovaries were sclerotic. Left tube contained a small cyst in the fimbriated end.

Aorta. — Both thoracic and abdominal portions were markedly atheromatous.

Bronchial nodes. — The right bronchial nodes were transformed into an irregular mass greatly infiltrated by lime salts.

Submaxillary node on the left side was enlarged and carcinomatous.

Supra-clavicular node on the left side was enlarged and carcinomatous.

MICROSCOPIC EXAMINATION.

Wrist. — The surface of the lesion is covered by a mass made up of pus cells, erythrocytes and fibrin, with here and there concentric masses of keratinized epithelium resembling the centers of epithelial pearls.

The surface epithelium is lacking in some areas, while elsewhere, although thinned, it possesses many irregular styloid-like processes extending into the underlying tissues.

In the subcutaneous area are many cell nests that vary greatly in size and shape. The peripheral cells in these nests are columnar in shape and arrangement and possess oval nuclei that stain comparatively deeply. As the center of the nest is approached the arrangement becomes very irregular, the nuclei become larger, lose their oval shape and stain faintly. In other instances the cells and nuclei become more elongated and flattened, are arranged in concentric layers and finally become transformed into masses of keratin. Between the cell nests are found many acini of the coil glands.

There is also a very extensive round cell infiltration throughout the tissue. The blood vessels are quite numerous and in many instances their walls are considerably

thickened. The cell nests are separated from each other by well marked areas of connective tissue. There is a large amount of elastic tissue fibers and fragments at the periphery of the growth, but it decreases greatly toward the central area.

Lip. — The specimen includes both skin and mucous membrane. The surface is covered by a thin layer of an exudate composed of pus cells, erythrocytes, and fibrin, excepting to one side where the surface epithelium remains. This latter suddenly increases very greatly in thickness and sends out prolongations that appear to be directly continuous with the underlying cell nests. The tissue is composed of innumerable small, narrow, and irregular collections of cells separated by strands of adult connective tissue. These cells differ extremely in regard to size, shape, and staining capacity. There are also similar differences in the nuclei. Some cells have undergone a slight keratin formation but no epithelial pearls were found. There is everywhere an extensive infiltration of small round cells and polymorphonuclear leucocytes. There are numerous blood vessels but excepting in the superficial zone there is not much congestion. Not many cells undergoing mitoses were found. There was a large amount of elastic tissue along the periphery of the growth and many individual fibers scattered throughout.

Cheek. — The specimen consists of a great many irregular and narrow cell nests separated from each other by a very small amount of connective tissue. There are marked differences in the size and shape of the cells, particularly in regard to the nuclei. These latter vary extremely in size, shape, and staining capacity. All degrees of staining from very deep to the mere shadowing of the nuclear wall are found. In many places the cell protoplasm has stained quite intensely with eosin, although the cells otherwise seemed normal. Some cells appear vacuolated, the nuclei having disappeared. There is some keratin with slight attempts at pearl formation. At the periphery of the growth are many

muscle fibers, some of which are atrophic, evidently from pressure. Fibers can also be demonstrated that have been surrounded by the infiltrating tumor cells. Cells undergoing division are not infrequent. In a few areas in the neighborhood of the muscle fibers there is seen a slight round cell infiltration. The blood supply is good and some congestion exists. There is comparatively little elastic tissue anywhere in the specimen. That which is present is found at the margin of the cancerous nodule. Within the tumor mass the elastic tissue is almost completely absent, although there is a considerable amount of connective tissue. There are no traces anywhere of glandular or lymphoid tissue.

Sub-maxillary lymph node (left). — The lymphoid structure has almost completely disappeared excepting here and there along the periphery adjacent to the capsule. The specimen is made up of a very extensive but irregular distribution of solitary cells and cell nests separated by very little connective tissue. There is a very great difference in size, shape, and staining capacity of both cells and nuclei. In many cells the protoplasm stains quite deeply with eosin. Many vacuolated cells are found. The blood vessels are not very large or numerous, nor do they show much congestion. A few fibers of elastic tissue are found in the periphery near the capsule and also a few isolated masses within the tumor growth.

Supra-clavicular node (left). — In this specimen there is nothing left showing the nature of the tissue excepting one small area of lymph cells and a thin connective tissue capsule surrounding the growth. The lymphoid tissue has been replaced by a mass of cells varying greatly in size and shape. The nuclei also differ greatly in their reaction to the stain, some taking it very deeply while others very faintly. There is very little connective tissue present; in many places it is apparently absent. Near the center of the specimen are several large areas of evidently degenerated tissue. They appear granular and although the general arrangement can

be made out yet no details are distinguishable. In these same areas are what appear to be acini lined by a layer of tumor cells. In these openings are found granules and cells in varying stages of degeneration. The impression made is that they are not true acini but are areas in which there has been a marked degeneration of the cells with absorption. In one small area in which there are numerous epithelioid cells are two very large masses of protoplasm in which is a great number of long oval nuclei situated chiefly near the periphery. These are apparently tuberculous giant cells. The elastic tissue has disappeared except at the periphery in the capsule, there being none in the tumor tissue.

Stomach.—The surface of the specimen is covered by a rather thick layer of necrotic tissue throughout which are found great numbers and masses of long mycelia-like structures. No bodies resembling spores were seen. Are probably saprophytes. There were also present great numbers of pus cells and lymphocytes. The tumor tissue is composed almost entirely of cells, the connective tissue existing merely as a few fine strands and fragments scattered irregularly. There is no definite alveolar arrangement, although in a few places the fibrous strands apparently enclose a few cells within an opening. The connective tissue could be distinguished only by the aid of Mallory's stain. The cells composing the neoplastic tissue show extreme polymorphism and variation in size. The nuclei may be round, oval, indented or very irregular. As a rule most of them stain chiefly at the periphery but many take the stain quite uniformly. In some are found one or more deeply staining bodies. The amount of protoplasm surrounding the nuclei is not very great in the majority of instances but, in some, forms the greater part of the cell. Many cells are found which contain two or more nuclei. In some places are seen nuclei that are apparently undergoing division as indicated by the irregular arrangement and the deep staining of the chromatin. The tumor cells have infiltrated between the muscle bands of the stomach wall, have widely separated

them and in many places completely destroyed the fibers. In a few places the cells are found within the connective tissue covering of the stomach. Many blood vessels are found throughout the tissue, some being quite large. The larger ones have distinct endothelial walls, but the smaller vessels are very incompletely formed. In many instances no endothelial lining can be distinguished. The entire specimen is everywhere greatly infiltrated with lymphocytes. There is a small amount of elastic tissue in the remnant of the stomach wall, but not in the tumor mass.

Gastric lymph node.— In the greater part of the node there is no lymphoid tissue remaining except in the peripheral zone close to the capsule. The new cells seem to have destroyed the original tissue; there being a comparatively sharp demarcation at the junction of the two forms. The new growth is composed of cells whose nuclei differ greatly in size and staining capacity. There is not as great a difference in shape as in the stomach tumor. The nuclei are comparatively round or oval and occasionally irregular, many stain but slightly while others are deeply colored. Here and there are found cells eight to ten times the size of most. The nuclei in them are very irregular and stain deeply. A few multinucleated cells are present. The amount of protoplasm in the cells is rather large. The amount of connective tissue is very slight and nowhere can an alveolar arrangement be seen. A few fibers between the cells can be made out with Mallory's stain. There are many blood vessels, the larger ones having distinct walls of endothelium which are lacking in the smaller. In one portion of the node there has been a very extensive hyaline degeneration of the connective tissue reticulum. In this area there are no tumor cells, the tissue being composed of lymphoid cells. There are also found several arrangements resembling tuberculous giant cells. There is a ring of oval or round nuclei situated at the periphery enclosing a slightly granular center that stains with eosin. In one section there was found one acid fast bacillus. A few epithelioid cells are present in the

neighborhood of the giant cells. A small amount of elastic tissue is present in the periphery underneath the capsule, but none amongst the tumor cells.

Liver. — The liver tissue shows much congestion and also cloudy swelling. In many places it has been completely replaced by a cellular mass in which there can be seen, here and there, a few isolated liver cells. The tumor cells vary greatly in size and shape. The nuclei also differ very much not only in size and shape but in their reaction to the stain. In many there are found small very deeply staining spots. Many of the larger cells contain two or more nuclei and, in some, evidences of mitoses can be seen. The amount of protoplasm varies greatly, in some cases there is a very slight rim while in others it forms a large mass. With Mallory's stain a few fine fibers of connective tissue can be seen here and there, but little if any true alveolar arrangement is present. In the capsule of Glisson between the lobules there is noted a very cellular infiltration, most of these cells resembling those found in the larger cellular areas, but there are many that resemble lymphocytes. Numerous neoplastic cells occur widely distributed throughout the liver in the capillaries. They can be readily distinguished from the other cells. There is also a slight hyperplasia of the bile ducts, some of which show considerable dilatation. No elastic tissue except that which surrounds the structures in the capsule of Glisson was found.

The conditions present in this case would seem to indicate that it was one of multiple primary tumors. According to the history the lesions of the lip and the wrist appeared simultaneously. In addition there were found a fibroma of the uterus and a multiple tumor formation of the stomach.

In regard to the gastric growth there are practically three possibilities as to its nature; it might be a primary sarcoma, a primary or a secondary carcinoma.

The microscopic examination of sections from the stomach, gastric lymph nodes, and liver reveals a structure that

very closely resembles that of sarcoma. The tissue is extremely cellular in type, there being in fact no discernible connective tissue fibers that in any way form alveoli. In between the cells can be found fine fibrils of connective tissue extending in all directions. Furthermore, in all these organs there are found many vascular spaces whose walls are formed either by the neoplastic cells or by an incomplete lining of endothelium. One, however, gets the impression that the cells do not resemble those that are usually found in sarcoma, they appear more like those of epithelial origin.

The second possibility is that the lesion is secondary in character and the result of implantation metastases from the primary growth on the lip. In support of this view the following can be stated. As has been shown by Moore¹ and Palmer² and by a series of examinations made by myself, there is a marked reduction or even absence of hydrochloric acid in the stomach in cases of carcinoma of other parts of the body. The lip lesion was much ulcerated, communicating with the inner mucous membrane, and consequently many particles of the carcinomatous tissue must have been constantly swallowed. These cells finding conditions in the stomach not unfavorable gained lodgment and grew. This would be indicated by the practically similar size of several, about three, of the lesions. There are, however, certain points that would incline toward a primary origin in the stomach. Out of two hundred and one autopsies performed in the Middlesex Hospital, London,³ on cases of carcinoma of the lip, mouth, and tongue, there were found no secondary deposits in the stomach. Out of one hundred and thirty-four autopsies at the same hospital for carcinoma of the lower pharynx and esophagus there were twenty-seven cases of secondary growths in the stomach and intestine; thirteen of the gastric tumors were the result of extension. The location of the other fourteen is not given. Reutter⁴ reports a case of double carcinoma of the esophagus and stomach in a man of sixty-eight years. Microscopic examination supported his contention but the variety of the growth is not given. Kaufman and Borst⁵ both mention a case

reported by Klebs in which there was a secondary carcinoma of the stomach resulting from the implantation of cells from a growth in the lower portion of the esophagus and which grew as a squamous epithelioma. Other similar cases are reported by Beck and Futterer.¹ Klebs also reports a case of secondary squamous tumor of the stomach following a similar growth of the tongue. Borst² mentions a case of squamous carcinoma of the stomach, the result of a metaplasia of the cylindrical epithelium. Futterer³ has shown by certain experiments that there can occur in the stomach of rabbits a metaplasia of the columnar epithelium into the squamous form.

In the instances of secondary growths above mentioned the type of the original tumor, the squamous variety, has been reproduced and preserved in the stomach. In this particular case now under discussion there has not been found at any time, and many sections have been examined, a trace of a squamous epithelial structure. There is no nest formation, no epithelial pearls and as far as can be determined no attempt at a keratin degeneration. If the growths were secondary to the tumor of the lip some traces of the original structure should be somewhere found in the stomach, gastric nodes, or liver.

The final claim is that we are dealing with a primary condition of the stomach with metastases to the adjacent lymph nodes and liver. The fact that there were multiple nodules in the stomach does not necessarily militate against the assumption that they were primary in origin. According to V. Hanseman⁴ there may be a pleuracentric origin of malignant growths. Borst¹⁰ in speaking of carcinoma of the stomach says that there may be not only several independent growths in that organ but that there may also be some in the intestine. He further remarks¹¹ that cases of multiple carcinoma of the stomach occur and that they probably result from metastases along the submucous lymphatics, but may be due to implantations. In connection with this Kaufman¹² speaks of metastases in the intestine in cases of carcinoma of the stomach being possibly the result of implantations.

Although the microscopic structure appears much like sarcoma it is probably carcinomatous. Kaufman¹⁸ describes a small round cell carcinoma of the stomach in the following: "the infiltration can be so diffuse that the alveolar formation can be distinguished only at the periphery." He speaks of one case that he saw in which there was "an enormous polymorphism, small and large cells with large single nuclei and other multinucleated giant cells being found in great number, so much so that the growth could easily have been mistaken for sarcoma (the latter, however, having large clear nuclei)."

From the above it would seem justifiable to consider this case one of multiple primary carcinoma of the wrist, lip, and stomach.

The possibilities of the lesions being either leukemic or tuberculous were also considered. It did not seem difficult to rule out these. There were no general enlargements of the lymph nodes and the spleen was small and atrophic, weighing only forty-five grams.

Sections of the stomach, gastric nodes, and liver were stained for tubercle bacilli and with one exception none were found. That was seen in the gastric node in the area that showed distinct tuberculous changes, none in the neoplastic tissue.

The coincidence of the carcinomatous lesion with the tuberculous changes is also a matter of interest but one that cannot at present be discussed.

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DESCRIPTION OF PLATE XXIV.

FIG. 1. — Multiple primary carcinoma of the stomach showing nodules and diffuse infiltration.

FIG. 2. — Diffuse secondary carcinomatous infiltration of the liver.



1



2

CONGENITAL RHABDOMYOMA OF THE HEART.*

REPORT OF A CASE ASSOCIATED WITH MULTIPLE NESTS OF
NEUROGLIA TISSUE IN THE MENINGES OF THE SPINAL
CORD.

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Only eleven cases of undoubted rhabdomyoma of the heart have been published. The most comprehensive paper upon this subject was published by Seiffert¹ in 1900, at which time nine supposed cases were on record. Seiffert rejected four of these cases; those of Kantzow and Virchow,² Skrzeczka,³ Rieder,⁴ and Yusti.⁵ He presented one new case and gave a careful review and summary of all the other cases. I have studied the original reports of the rejected cases and agree with Seiffert in the opinion that the tumors described were not rhabdomyomata.

Seiffert's paper clearly showed that the rhabdomyomata of the heart are all of one type. All of the cases collected by him have agreed very closely in gross appearances and in histological structure.

Since the publication of Seiffert's paper five new cases have been recorded. The case which I shall describe is the twelfth. All of these later cases (with the possible exception of Rothe's,⁶ which has not been fully reported), including my own, are similar to those collected by Seiffert.

It seems advisable to assemble again the cases of rhabdomyoma of the heart because of the additional evidence of their congenital nature, their association with diffuse scleroses of the cerebral cortex, and the frequent association with disturbances of general nutrition. Each new case is therefore of interest from the casuistical view-point.

* Received for publication June 1, 1907.

Mesenteric nodes are very prominent; pale and firm. On section are uniformly pale pinkish yellow, the largest are about one centimeter in diameter. Appendix is three centimeters long, directed upwards and inwards and has a mesentery to its tip. Fold of Treves present.

Chest. — The thymus is small, represented by two rather broad, thin lobes of loose textured, soft, lobulated gray tissue. Extends upwards one centimeter above innominate vein; inferiorly it reaches half way down over pericardium. Weight, four grams.

Pleural cavities. — Normal. No adhesions; no free liquid.

Pericardial cavity. — Normal; contains about three cubic centimeters of clear, very pale yellow liquid. The ductus arteriosus is closed.

Heart. — Weight, 72.5 grams. Very large. Pulmonary artery opened in situ contains much dark red liquid blood and a small amount of cruor and chicken fat clot. The right ventricle is markedly distended with liquid blood, and cruor clot. The left ventricle contains a small amount of elastic grayish, non-adherent clot. In the right ventricle, below the posterior segment of the pulmonary valve, is a spherical tumor, 1.7 centimeters in diameter. It is attached to the papillary muscles of the left anterior segment of the tricuspid valve. From the right side of the tumor mass a group of cordæ tendinæ emerge to become attached to the right anterior segment of the tricuspid valve. The tumor thus takes origin from the interventricular septum. The tumor on the upper surface has an indentation forming a small mound-like projection, four millimeters in diameter. The color is grayish red and several shades lighter than the myocardium. Consistence is firm and elastic. On section the surfaces appear very slightly translucent. The tumor extends into the myocardium for a depth of three or four millimeters, so that the real shape is ovoid. The long diameter is two centimeters. It is sharply separated from the myocardium by a pale whitish line. The distance below the level of the pulmonary valve is three millimeters. The position of the tumor, in relation to the tricuspid valve, is directly opposite the junction of the right and left anterior segments (Fig. 1). The left side of the heart is markedly dilated. Length from auriculo-ventricular groove to apex is six centimeters. The greatest breadth is six centimeters. The

papillary muscles are markedly hypertrophied. The left side of the heart is small. Valves and endocardium are normal. Foramen ovale is closed. Myocardium is firm, brownish red. There are a few injected points over external surface of auricular appendages and at base of pulmonary artery.

Measurements: Tricuspid valve, 5.2 centimeters.

Pulmonary valve, 4.6 centimeters.

Mitral valve, 4 centimeters.

Aortic valve, 3.3 centimeters.

Left ventricle, thickness of wall, .6 centimeter.

Right ventricle, thickness of wall, .5 centimeter.

Lungs. — Both lungs are similar in appearance; are pale pink; of slightly increased consistency and crepitant throughout. At posterior borders there are a few dark red punctate areas. On section cut surfaces are moist and yield a large amount of dark red liquid blood. Both lungs are air-containing throughout. Section of dark red punctate areas show miliary sized dark red foci. Bronchi and vessels are normal. Bronchial nodes are not remarkable.

Spleen. — Weight, thirty-seven grams. Dark red. Consistency firm and elastic. Capsule smooth and glistening. On section cut surfaces are dark red and smooth. Glomeruli and trabeculae visible. In the gastrosplenic omentum is a small accessory spleen .5 centimeter in diameter.

Gastrointestinal tract. — Normal. Mucosa is everywhere pale. Stomach contains a small quantity of acid smelling liquid. Pylorus, 1.8 centimeters in circumference. Contents of duodenum are smooth and bile tinged. The small intestines contain a very small amount of non-offensive, pasty, yellowish material. The colon contains a small amount of smooth greenish yellow non-offensive feces.

Pancreas. — Normal. Grayish pink and firm with well defined borders and lobulations.

Liver. — Weight, three hundred and eleven grams. Brownish red in color. Surfaces smooth; consistency normal. On section lobulation not visible. Gall bladder contains a small amount of transparent, greenish bile. Ducts are patent.

Kidneys. — Weight, 81.5 grams. Are large. Ureters are dilated. Surfaces are injected. On section pelves of kidneys contain thin, turbid liquid. Both poles of both kidneys are mottled dark red and yellow. Cut surfaces show radial yellow streaks surrounded by narrow injected zones. These are found throughout the substances of both kidneys but are most marked at the poles. Cortex pale, rather opaque gray with injected radial vessels. The pyramids are deep red and contain many yellow

streaks and dots. The pelves are deeply injected. The ureters are injected and contain yellow turbid liquid. The bladder is contracted and contains a very small amount of yellowish turbid liquid. The mucosa is thrown into vertical, dark red, velvety folds.

Adrenals. — Normal.

Aorta. — Normal.

Retroperitoneal lymph nodes. — Are large, pale and firm. Are particularly abundant along aorta and below coeliac axis.

Thoracic tract. — Normal.

Organs of neck. — Not removed.

Bones. — Costo-chondral junctions are markedly enlarged. Sections show soft red areas in the bone adjacent to line of cartilage and bone junction. The muscles of the thighs are edematous and of brawny consistency. The deep layers are stained yellowish and red. On incising to obtain bone marrow the periosteum is found separated from the bone by a layer of dark red blood clot. The left femur removed entire; hardened in Kaiserling. Frontal section made. The periosteum is everywhere separated from the bone by a layer of soft blood clot, thickest at lower end just above epiphysis and here the layer of clot is one centimeter deep. It gradually decreases in width to the upper epiphysis where the periosteum lies in contact with the bone. The lower end of the shaft is severed from the cartilage at the epiphyseal line by a narrow hemorrhagic zone. The upper end of the shaft is trabeculated and dark red. The marrow is very abundant and completely fills the shaft; is opaque pinkish gray in color. The center of ossification, in the head, is three millimeters in diameter; the lower center is fourteen millimeters transversely; eight millimeters vertically. The upper edge of the latter is dark, hemorrhagic in places.

Head. — Hair very pale; rather coarse in texture and sparse. Over the posterior vertex the scalp is nearly denuded of hair. The hair here is very short; in places visible as dark points, giving the effect of having been closely clipped or recently shaven. The scalp is normal. Fat layer is two to three millimeters deep. The anterior fontanelle measures eight by three centimeters. Posterior fontanelle closed. Frontal suture is united inferiorly; the upper half is ununited. Other sutures are ununited. The edges of the lambdoidal and coronal sutures are deep red. Bones of calvarium are thin and brittle. Sinuses are normal. Dura normal, strips readily from calvarium and base of skull. There is a very large amount of clear liquid, one to one and a half centimeters deep, between pia and dura. The pia is normal.

Brain, consistency of the tissue is firm; the hemispheres as a whole are fluctuant. The convolutions appear to be very numerous and small. The sulci are broad. The appearance differs markedly from the normal brain in apparent number and smallness of size of the convolutions. Brain hardened in toto in ten per cent formalin, for future study. Examination of hardened brain shows apparent smallness and number of the gyri to be due to vascular grooves; this becomes apparent after stripping the pia. The lateral, third and fourth ventricles are markedly

dilated. The roof of the fourth ventricle posterior to the cerebellum (*i.e.*, posterior velum interpositum) bulges outwards. Section shows an apparent excessive amount of choroid plexus.

Middle ears. — Normal.

Spinal cord. — The spinal cord above tenth dorsal vertebra removed posteriorly by sawing through laminæ. This portion of the cord is of uniformly firm consistence. The dura is loose, normal. On the dorsal surface of the cervical enlargement are many minute, barely visible, translucent elevations, giving a finely sanded appearance to the pia-arachnoid. Nothing abnormal found on section of the cord. The spine below the tenth dorsal vertebra including sacrum and coccyx removed in toto. The spinal canal and sacrum are exposed by removing the anterior wall and very careful sectioning of the laminæ. The dorsal surfaces of the upper half of the sacrum, the fifth and possibly fourth lumbar vertebrae are open. The coccyx and tip of sacrum are closed. The open portion of the sacrum apparently is that portion formed by the two upper segments. Opposite this on the dorsal surface the cord runs into a cavity about three centimeters in diameter. This cavity is lined with a pearly white and pink membrane. The cord fuses into the upper dorsal portion. The diameter of the cord at this point is about half that of the mid-dorsal cord. The wall of the sac is traversed by numerous small nerves coming from cord and from a terminal filament which runs into the midline of dorsal surface of sac. Gross specimens saved in Kaiserling.

ANATOMICAL DIAGNOSES.

Internal and external hydrocephalus; lumbo-sacral rachischisis; acute cystitis; pyelonephritis; rhabdomyoma of heart; pulmonary stenosis; tricuspid insufficiency; scorbutus.

MICROSCOPIC EXAMINATION.

Heart muscle from wall of left ventricle including one of posterior group of papillary muscles. Negative.

Lung. — Moderately injected. Large groups of alveoli are filled with red blood corpuscles and compact granular or reticular masses of hyaline material which stain deeply with eosin. The proportions of hyaline material and corpuscles vary. In many alveoli where both are present the corpuscles are pale, swollen, and each one is surrounded by deeply staining eosinophilic granules. Various stages of disintegration of the red corpuscles may be seen and it is evident that the hyaline material is derived from the red corpuscles. The cellular reaction in the alveolar walls corresponds with the age of the processes in the individual alveoli. The alveolar walls in general are thickened. Where the alveoli contain chiefly red blood corpuscles and lightly staining hyaline material there are numerous large mononuclear cells with abundant protoplasm and large oval or round vesicular nuclei within the capillaries and beneath the alveolar epithelium. These cells correspond to endothelial cells in all respects. A few are phagocytic. There are also a few cells with less

abundant, basic staining protoplasm and nuclei characteristic of the lymphoid series, *i.e.*, with peripherally arranged chromatin granules. In alveoli which contain compact deeply staining masses of hyaline material, there is also occasionally actual fibrous tissue increase in the alveolar walls, demonstrated by the phosphotungstic acid hematein stain. All of the alveoli containing foreign material contain also many large mononuclear phagocytic cells, surrounding and invading the hyaline material. These phagocytic cells are very large, ameboid in shape, many contain hyaline droplets stained with eosin, a few contain small amounts of dark brown granular pigment. There are a very few cells with two and three nuclei. A few polynuclear leucocytes are in the alveoli and vessel walls. There are numerous atelectatic alveoli with thickened walls, and filled with large vacuolated phagocytic cells which contain hyaline material and brown granular pigment. The alveolar walls in such places contain many large irregularly polyhedral cells with basic staining protoplasm and large nuclei with a coarse basic staining reticulum and peripherally arranged protoplasm. These cells resemble certain myelocytes in the bone marrow.

The larger blood vessels are normal, and contain normal blood.

Bronchi are normal.

Spleen. — Markedly injected. No hemorrhages. The follicles are numerous, of fair size. The centers are occupied by a coarse connective tissue reticulum in which are a very few lymphoid and large pale often degenerated cells resembling endothelial cells. The lymphoid tissue is thus actually reduced in amount. The pulp contains numerous lymphocytes with abundant basic staining protoplasm and nuclei like those of plasma cells. A few of these cells show mitotic nuclei. There is a small amount of pale brown granular pigment in the cells lining the sinuses and about the trabeculae. Vessels and capsule are normal.

Liver. — Moderately injected. Fat vacuolation not evident. Liver cells contain a very small amount of pale brown granular pigment. There are a few small areas of necrosis scattered without special localization in the lobules. The smallest areas consist of a few poorly staining, vacuolated liver cells containing hyaline droplets; the larger areas contain a fibrin-like reticulum and many polynuclear leucocytes. The sinuses throughout the liver contain occasional cells resembling myelocytes and which have little granular basic staining protoplasm and large vesicular nuclei containing a coarse reticulum and large irregularly arranged chromatin granules.

Gall bladder. — Negative.

Pancreas. — Negative.

Small intestine. — Negative.

Colon. — Negative.

Appendix. — Negative.

Kidney. — Markedly injected. There are many large foci of suppuration consisting of compact masses of leucocytes and remnants of kidney structure. These foci are situated in both cortex and medulla. Some are elongated and extend from medulla to cortex. The leucocytes are

largely polymorphonuclear; there are also many larger neutral staining cells with indented or lobated nuclei. There is marked cloudy swelling of the kidney tissue remote from the necrotic foci. Many tubules in the tissue adjacent to the abscesses are packed with polynuclear leucocytes. In the pyramids there are large collections of lymphoid and plasma cells in the interstitial tissue. There are similar collections about the blood vessels. Portions of the cortex show but slight change in the tubules while the glomeruli are often normal, though of infantile type. The pelvic epithelium is infiltrated with polynuclear leucocytes. The underlying tissue is edematous and infiltrated with polynuclear leucocytes, lymphoid and plasma cells.

Adrenal. — Negative.

Urinary bladder. — The submucosa is edematous in places and is infiltrated with lymphoid and plasma cells and a few polynuclear leucocytes. In places there are hemorrhages just beneath the mucosa, which itself contains many red blood corpuscles between the epithelial cells.

Skin and fat tissue from abdomen. — Epidermis and corium are negative. The fat cells are those of normal fat tissue.

Ovary. — Contains many primitive ova and a few Graffian follicles which are visible to the unaided eye and about .5 millimeter in diameter.

Marrow from femur. — Very cellular with evidences of great erythroblastic and leucoblastic activity. Not otherwise remarkable.

Aorta. — A section taken from thoracic portion just below the arch shows a thickening and degeneration of the intima for about a third of the circumference. The thickened intima in places has diffuse basic staining areas with loss of detail in the cells. There are also a few areas with a loose bluish staining fibrous meshwork among which are a few active connective tissue cells. The media shows slight hyaline change of smooth muscle fibers in a few places beneath the thickened intima.

Lymph nodes. — From mesentery and along aorta show a moderate degree of activity.

Thymus. — Contains fewer cells than is normal at this age. The differentiation of cortex and medulla is absent. The Hassal bodies are not remarkable in number or structure.

Costochondral junction. — The line between cartilage and bone is very irregular. In places the cartilage has been replaced by a loose reticular tissue, resembling embryonic connective tissue, without the formation of osteoid tissue. There is no definite layer of cortical bone close to the cartilage; the inner layer of the periosteum is active and contains irregularly arranged trabeculae of osteoid tissue, some parallel, others perpendicular to the long axis of the shaft. In the medulla of the bone are large areas of hemorrhage. Portions of these areas are of long duration and contain many brown pigmented cells. Into these areas and at the periphery there are irregularly arranged deposits of osteoid tissue and occasional nests of cartilage with osteoid tissue forming at the peripheries.

Periosteum and cortex of femur. — The clot separating the periosteum from the bone consists almost wholly of red blood corpuscles with very

little fibrin and hyaline material. The clot is traversed in all directions by delicate capillaries. At the edges of the clot on both sides are irregularly arranged trabeculæ of osteoid tissue. The layer of osteoid tissue about the cortical bone is much less abundant than that from the periosteum and seems to come from tissue once connected with the endosteum, as the cortical layer of bone is imperfect. From the periosteum there is a marked very cellular ingrowth of connective tissue cells and capillaries. In places there is very abundant formation of osteoid tissue, and an occasional nest of cartilage cells surrounded by cells similar to those surrounding the trabeculæ of osteoid tissue.

Muscle from thigh.—The muscle bundles are widely separated by edema. A few fibers show hyaline change and loss of striations.

The heart tumor.—At the autopsy a vertical slice two millimeters thick was taken through the center of the tumor, going down into the heart muscle at the base for a depth of two to four millimeters. This was placed immediately into Zenker's fixative.

Sections through one-half of this piece show that the tumor is sharply outlined from the heart muscle by an almost continuous layer of connective tissue of varying thickness. In places this connective tissue layer is not thicker than the septa separating the bundles of muscle in normal heart tissue in regions close to the endocardium. In a few places it is entirely absent. The average thickness is about that of the layer of the normal endocardium of an infant of the same age. The free surface of the tumor is covered by a layer of compact connective tissue of fairly uniform thickness which is continuous with the endocardium of the heart wall, and like it covered with endothelium. In the connective tissue separating the tumor from the myocardium there are a few scattered atrophied muscle fibers. Running radially inwards from the connective tissue capsule are heavy trabeculæ of dense connective tissue, which carry blood vessels of large size. From these trabeculæ there are given off more delicate, less dense connective tissue processes which ramify everywhere through the tumor. There is an abundant supply of blood vessels to all parts of the tumor.

The low power picture of the tumor is striking. It consists of a loose sponge-like tissue with empty spaces of irregular

sizes and shapes. Apparently between the spaces are large cells which show extreme variations in size and shape. A few of the spaces seem to contain smaller cells, with many processes running to the walls. Here and there are connective tissue septa carrying blood vessels, while numerous capillaries accompanied by very little connective tissue are found in all parts of the tumor between the cells or empty spaces (Fig. 2).

With high powers, using ordinary stains, many of the spaces can be seen to be surrounded by a thick wall of protoplasm with a curved nucleus lying in a portion which is generally thicker than the rest of the wall. There are occasional deeply stained eosinophilic granules in the protoplasm and often these are orderly arranged in rows to form transverse striations of the protoplasm. Lying between the spaces there are a very few elongated cells with large oval nuclei and definitely though very finely fibrillated protoplasm. The cells with many processes lying within clear spaces often show irregularly and regularly arranged eosinophilic granules. The processes which run outwards are often transversely striated with rows of delicate eosinophilic granules. The processes merge into the walls of the spaces in a very perplexing fashion, sometimes they seem to end abruptly, at other times running obliquely they appear to fuse with the walls. Occasionally the walls of the spaces show very definite cross striations. Most of the cells have a single nucleus, many have two and three nuclei; rarely there are four nuclei in a single cell.

The spaces are generally absolutely empty. A few contain granular detritus or a circular reticulum of faintly staining material. Mallory's connective tissue stain helps to make clear the locations of the empty spaces. With it there is demonstrated a delicate connective tissue reticulum, hitherto unsuspected, running between the cells and the spaces. The acid fuchsin brings out the cross striations very clearly and it can now be seen that the walls of the clear spaces consist of cross striated protoplasm, continuous with the protoplasm surrounding an eccentrically situated nucleus or with

the processes radiating from a central protoplasmic mass containing a nucleus. The clear spaces lie within the cell. Their walls are composed of muscle protoplasm usually showing cross striations. The connective tissue stroma does not enter into the formation of these spaces (Figs. 5 and 6).

The tumor cells vary greatly in size and shape. The size depends much upon the size of the cavity within the cell. The great variety of shapes of the cells, some of them very grotesque, make measurements uncertain and unsatisfactory. The smallest cells are almost round or oval in shape and range upwards in size from thirteen microns in diameter. The largest cells, traced out by serial sections, reach a size of two hundred by one hundred and fifty microns. The largest number of the tumor cells range from one hundred by fifty microns to one hundred and sixty by eighty microns.

The nuclei are more constant in size and characteristics. They range in size from seven by nine microns to nineteen by nineteen microns. The majority of nuclei, however, lie between the limits of nine by seven microns and thirteen by ten microns. Where there are several nuclei to a cell they are apt to be smaller than when single, but this is not constant. The largest nuclei are in large cells and show evidences of preparation for direct division. No mitotic nuclei were found.

The nuclei are sometimes surrounded by clear spaces. They have distinct and sharply staining membranes. They contain usually one large spherical nucleolus which, with proper differentiation after the methylene blue and eosin stain, retains a purplish color. The nucleoli occasionally show the vacuolation commonly seen in ganglion cells of the central nervous system and are often five to six microns in diameter. Sometimes there are two or three nucleoli to a nucleus. Occasionally irregularly outlined or constricted nuclei are found which have two or three nucleoli and the chromatin granules grouped into two or more masses. This picture is considered to be evidence of direct division. The nuclear network is fairly coarsely meshed. The chromatin granules are small and irregularly distributed.

The cross striations and finer cell structures were best studied with Mallory's phosphotungstic acid hematein stain, as with it many unsuspected details were brought to light. It clearly shows that the walls of the spaces described contain striated fibrillæ, analogous to the fibrillæ of normal heart muscle. It can often be shown that these fibrillæ take origin or pass through the mass of protoplasm surrounding the nucleus. Very few fibrils were found which exhibited the alternate broad and narrow cross striations of normal heart muscle. Most of the fibrillæ consist simply of deeply staining blue dots connected by a very delicate fibrillary material which stains brownish. The striated fibers from adjacent cells in rare instances intermingle. They may run directly between two cells or join from two or more cells to form collections of parallel fibrils (Figs. 3 and 4).

In the large cells with abundant protoplasm there are often clusters of deeply staining granules without any definite arrangement or connection with one another. There is sometimes an arrangement in pairs of these granules, which are then biscuit shaped with the flat sides apposed, giving an appearance strongly suggestive of centrosomes. The granules when stained with the connective tissue stain are colored a brilliant red with the acid fuchsin. Here and there in the cells containing these granules we find groups of two to many connected by very delicate fibrillæ in a more or less orderly fashion. This is evidently the beginning of the muscle fibril formation. The delicate fibrils connecting these small bodies, when the phosphotungstic acid hematein stain is used, are stained brownish; with the connective tissue stain they are colored blue.

In attempting to orient these details and to find analogies with the normal muscle structure I was much aided by an observation by Professor Mallory made several years ago and which has not been published. Mallory found that the connective tissue stain stains the sarcous elements red and the delicate striations between the sarcous elements, that is, the membranes of Krause, blue. This is what is to be expected if these striations are of the nature of a binding substance.

In the tumor cells stained with the connective tissue stains the delicate fibrillæ connecting the fuchsin stained bodies are stained blue. With the phosphotungstic acid hematein stain they stain brownish. These facts justify the belief that the granules of the tumor cells, at first irregularly arranged and later assuming more or less of an orderly arrangement, are primitive sarcous elements. It also becomes evident that the orderly transversely arranged dots most prominent in the tumor cells and cell processes are primitive sarcous elements. The fibrillary material taking the blue of the connective tissue stain is to be regarded as a material similar to that of Krause's membrane in normal muscle. In a few places in the tumor the differentiation is more complete and we have alternating thick and delicate striations,—the sarcous elements and the membranes of Krause in normal muscle (Figs. 7, 8, and 9).

Occasionally in some of the large tumor cells there are non-striated deeply staining fibrils, which can be demonstrated with the phosphotungstic acid hematoxylin stain. These fibrils run in all directions through and upon the surfaces of the cells. They resemble neuroglia fibrils in size and in staining reaction. They give rise to pictures suggestive of neuroglia cells found in gliomata. Both striated and non-striated fibrils may occur in the same cells.

The non-striated fibrils at first thought are difficult of interpretation, but the evidence of the double differentiation of the tumor cells into the homologues of the contractile and binding substances of normal muscle make it probable that the non-striated fibrils represent an excessive formation of the binding material (Fig. 10).

Many of the tumor cells contain large spherical drops of hyaline material, which are probably preliminary to the formation of vacuoles. The hyaline material often lies completely surrounded by curved striated fibrils. Similarly arranged fibrils enclose larger volumes of granular material, while many of the largest spaces in cells contain considerable granular material.

Spinal cord and brain.—Special care was given to the

examination of the brain in view of the possible association with diffuse gliosis, even though the cortex was macroscopically not suggestive of such a process. Two pieces were taken from each of the following regions: frontal, parietal, paracentral, occipital and temporal, and from the superior surface and worm of the cerebellum. The sections were stained with hematoxylin and eosin and by Mallory's method for neuroglia fibrils after formalin fixation.

The cerebral cortex from the above locations proved to be practically normal. The number and arrangement of ganglion cells appear to be normal. Some alterations probably exist as a result of the internal hydrocephalus, but these are too slight to be demonstrated except by a series of comparisons with the normal infant brain. The amount of neuroglia is not above normal and is not as great as that of the normal adult brain. In the sections from the parietal cortex two nests of neuroglia were found, one in the substance of the cortex, the other in the pia surrounding a small blood vessel. These isolated findings will be discussed below in connection with similar neuroglia nests found in the meninges of the spinal cord.

Spinal cord.—With ordinary stains the cord appears normal. Mallory's neuroglia stain reveals a rich diffuse neuroglia network throughout the cord at all levels examined. There is probably a slight general increase of the neuroglia in spite of the even distribution. The amount exceeds that found in the normal adult cord, and an occasional active neuroglia cell is found in the subpial tissue and in the gray matter. A stain for myelin sheaths shows a scarcity in the mid-dorsal and lower cervical cord in the column of Burdach, otherwise there does not seem to be a deficiency in nerve fibers.* The ganglion cells in the gray matter, though surrounded by a heavy feltwork of neuroglia fibers, are normal in appearance, with abundant large Nissl bodies and normal nuclei and processes. The Marchi method

*The method employed for staining myelin sheaths was the iron hematoxylin method after formalin fixation and frozen sections; a modification employed by Professor J. H. Wright.

for fat shows the presence of slight to marked fatty change in the sheaths of isolated fibers or in groups of two to four fibers scattered uniformly, though sparsely, throughout the white matter of the dorsal and cervical regions.

In the meninges of the cord beneath the dura there are a great many small discrete nests of neuroglia tissue. They are most numerous on the dorsal surface of the cervical cord and produce the sanded appearance noted at autopsy. The number gradually diminishes towards the inferior end of the cord and in sections of the tenth dorsal segment there are very few. In the cervical cord a few are found upon the ventral surface.

These neuroglia nests are not connected with the spinal cord. This was determined by three sets of serial sections made from different levels of the cord. The nests lie beneath the arachnoid. A few are partially imbedded in the pia, but none are connected with the cord. All are invested with a very delicate sheath of connective tissue derived from the pia or arachnoid. They are irregularly spherical, ovoid, and rod-like in shapes. The largest nests partly ensheath the cord and are disc-shaped with rounded edges, often with extensions from the periphery. In size the largest are one to two millimeters by .2 to one millimeter by .1 to .3 millimeter. The greater number of these nests are spherical or ovoid in shape and range between seventy and one hundred and forty microns in diameter. Many of these nests lie in contact with blood vessels, and some surround small vessels; all of them contain strands of fibrous tissue derived from the processes of the pia and arachnoid. Occasionally they are attached to a vessel by a stalk-like process of connective tissue with or without a capillary (Fig. 11).

Aside from the connective tissue and blood vessels, which by serial sections can be proven to come from the pia or arachnoid, these nests consist of pure neuroglia tissue. They consist of a dense feltwork of fibers which run in all directions, but usually conforming to the long axis of the nest. Many have a central mass of concentric fibers. At the edges

there is usually an arched arrangement of the fibers similar to that at the surface of the normal spinal cord. The nuclei are invariably in the central portion and differ in no way from the normal neuroglia nuclei found in the central nervous system. No evidences of nuclear activity were found in these nests but occasional neuroglia cells with abundant protoplasm were found.

A few posterior nerve roots contain small similar nests of neuroglia tissue, always adjacent to a small blood vessel. Several dorsal root ganglia examined are normal. On the superior surface of the posterior velum interpositum are large convoluted nests of neuroglia similar in structure to those in the meninges of the cord. Two of these nests were found containing spaces lined with ependyma. One of the nests is connected with the cerebellum. In the floor of the fourth ventricle are several ependyma-lined canals and isolated groups of ependymal cells.

In the interpretation of the characteristics of these neuroglia nests we are forced to the conclusion that they are developmental in origin. The absence of any connection with the spinal cord is proven. Their structure with a central mass of cells speaks against the probability of their being outgrowths of normally existing neuroglia. The finding of similar nests containing ependyma is additional proof of the developmental origin.

A careful search of the literature of the brain and spinal cord has failed to reveal any record of similar isolated neuroglia nests in the meninges. The two nests found in the parietal cortex are to be regarded as similar to those from the cord and in no way allied to the diffuse sclerosis described in association with rhabdomyomata.

The cases of rhabdomyomata reported before Seiffert's publication have been reviewed by Seiffert. These are the cases of von Recklinghausen,⁸ Virchow,⁹ Hlava,¹⁰ Kolisko,¹¹ and Caseris-Demel.¹² One other case not mentioned by Seiffert is that of Billard, which Kolisko mentions in his

paper. Billard's case occurred before the days of histological examinations, but probably was a case of multiple congenital rhabdomyomata, for there were three small tumors in the anterior wall of the left ventricle and in the interventricular septum in an infant three days old. This case was published according to Kolisko in "*Traite des maladies des enfans nouveau-nés et à la mamelle*, Paris, 1828," which is not accessible to me. The same case is mentioned by Virchow in "*Die Krankhaften Geschwülste*, Bd. iii, p. 99."

The points which have interested most of those who have studied these tumors are the histology and particularly the nature of the spaces, the resemblance of the tumor cells to embryonic heart muscle, and in later cases the association with sclerosis of the cerebral cortex.

Von Recklinghausen's report was a very short one. He briefly described the character of the tumor cells and called attention to their similarity with embryonic cells described by Weismann in lower vertebrates. He arrived at no conclusion about the spaces, and although he stated that the spaces had no lining cells he considered the possibility of their being lymph spaces (similar to the macroglossia of Virchow) or blood spaces or muscle tubes of pathological origin. Cerebral sclerosis was noted by him, though not described, in the following words: "*im Gehirn war ferner noch eine grosse Zahl von sklerosen vorhanden.*"

Virchow did not mention the head in his report (the autopsy was done by Susewind of Braunsfels and Herr of Wetzlar). As possibilities for the nature of the spaces he mentions lymphatics and edema.

Hlava's case was one with a single tumor having the characteristic histology. The head is not mentioned. Hlava believes the spaces to be due to the effect of alcohol fixation and regards them as intracellular.

Kolisko reviews the cases preceding his and like Seiffert rejects the cases of Kantzow-Virchow and Skreczka. Nothing abnormal is noted in the brain in Kolisko's case. His gross and microscopic description of the tumors is very detailed and agrees closely with later descriptions. The

spaces are regarded by him to be between the tumor cells and they are so drawn on a plate accompanying his paper.

Caseris-Demel was the first to call attention to the many processed cells lying within spaces and called by him spider-like cells. He regards the spaces to be intercellular and similar to spaces which he found between anastomosing cells in the hearts of human embryos. He reports cerebral sclerosis associated with his case but does not describe the condition, except to make the statement that it was not inflammatory. There were also small nodules in the kidneys composed of nests of embryonic renal tissue without glomeruli.

Seiffert's paper includes a very complete discussion of all the preceding cases. His description of the tumors is very complete and accurate and agrees very closely with my case. He proved satisfactorily that the spaces are intracellular, and calls attention to the many processed cells, which he likens to spiders in their nets. The cells with the spaces he compares to huge embryonic muscle cells. The striations are made up of granules whose nature he does not distinctly specify. He draws comparisons between the tumor cells with spaces and embryonic heart muscle cells during the stage of fibril formation where the fibrils are limited to the periphery of the cell while the central portion is filled with a homogeneous substance which does not stain.

Rothe incompletely demonstrated a case of brain sclerosis associated with multiple tumors of the breast. The brain lesions were typical of the multiple tuberous form of sclerosis associated with idiocy by French observers. The tumors of the heart he believes to be rhabdomyomata, without however offering histological evidence. As this was demonstrated before a society, and no further report has appeared, I am unable to pass judgment upon this case, except to accept Rothe's opinion and to agree in accepting the macroscopic characteristics as evidence of the tumors being rhabdomyomata.

Fordick²¹ was the first to call attention to the association of rhabdomyoma of the heart and cerebral sclerosis. He

reports two cases of multiple rhabdomyomata with diffuse cerebral sclerosis. Histologically his two cases are similar. The heart tumors have the characteristic structure of previous cases. He does not give minute descriptions, though he calls attention to the resemblance of the tumor cells to embryonic heart muscle. He regards the spaces as intracellular in position. He believes the tumors and the sclerosis to be congenital and decides that the two conditions are in some way related. The type of sclerosis in these two cases is a diffuse fibrillary gliosis limited to the gray matter. No fine histological details are given though the gross distribution of the lesions is accurately indicated.

Bonome¹⁴ has described two similar cases of diffuse cerebral sclerosis, one of them associated with multiple rhabdomyomata of the heart. The type of sclerosis is a diffuse fibrillary gliosis with degrees of atrophy and degeneration of the nervous element corresponding to the degree of gliosis. The process is an active one, though evidently of intra-uterine origin. The histological details of the rhabdomyomata are characteristic, though Bonome differs from other observers and myself in the interpretation of some of these. He distinguishes two kinds of tumor cells. Type one, found at the centers of the nodules, consists of large, round or polygonal cells with one to three nuclei. The cells are often vacuolated and often contain hyaline bodies as well as small granules which stain intensely with fuchsin. These fuchsin staining granules he thinks are products of degeneration. The cells of this type usually have many processes which branch and run into the walls of the spaces. These cells he considers to represent embryonal muscle cells. The second variety of cell is found at the peripheries of the tumor nodules. They are larger than the cells of type one, more regular in shape and show less tendency to degeneration. The nuclei are less vesicular than those of type one and show evidences of direct nuclear division. These cells also have more processes than the cells of type one. The processes show cross and longitudinal striations while the bodies of the cells show more marked striations, both cross and longitudinal,

than do the cells of type one. The reasons for nuclear activity seem to be chiefly the smaller size and great richness in chromatin of the nuclei. The cells of the second type Bonome believes represent more nearly normal myocardial cells.

He believes that the spaces in the tumor lie between the tumor cells and the connective tissue stroma, and that they are formed by the degeneration of cells of type one.

Both of Bonome's cases of cerebral sclerosis were in rachitic infants. He believes the association of rhabdomyoma and cerebral sclerosis to be dependent upon the same conditions in intra-uterine life, namely disturbances of nutrition and resulting vascular lesions. The neuroglia tissue being more resistant than the nerve cells survive the condition of defective nutrition. The rhabdomyomata develop from embryonic muscle fibers which become isolated through a connective tissue overgrowth replacing adjacent degenerated muscle fibers.

The case of Knox and Shorer¹⁵ was the first case to be reported from this continent. Their microscopic description is short and agrees with the other cases. They believe that the spaces are intracellular but do not offer additional evidence. They call attention to the similarity of the tumor cells with the Purkinje cells and the cells making up the conducting bundles in the heart. The head is not mentioned.

The location of the spaces in the tumors may be considered to be definitely determined within the cells of the tumor. Seiffert's evidence in favor of this point is very convincing while the evidence revealed by the phosphotungstic acid hematein stain is conclusive. Nearly every observer studying these tumors has pointed out the analogies between these cells and embryonic heart muscle. Seiffert and Knox and Shorer have laid stress upon the clear spaces in the embryonal cells and in modified muscle cells found in adult hearts. I have seen cells similar to those they describe in a moderator band from an infant's heart. Large vacuolated cells are not

rare in the myocardium of infants which have died from acute infectious diseases. In a case* of general anasarca due to heart and kidney disease there were large hollow muscle cells in the heart evidently due to edema. The muscle fibrillæ forming the walls of these cells appear normal. The vacuolated condition of these cells suggests a possible origin of the spaces in the tumor cells. Another possible origin is the disintegration of the hyaline drops and a replacing of the same by increasing amounts of serous liquid, conceivably by processes following autolysis.

The congenital nature of these tumors is established by their occurrence in new born infants and the failure to find them in children over three years of age. With the exceptions of Hlava's and my cases the tumors have been multiple. Ponfick in studying his cases came to the conclusion that the large and small tumor nodules were of the same age, because of their perfect similarity in structure. This fact together with the invariable occurrence in infants led him to the decision that they are congenital.

The most interesting fact in the general considerations of rhabdomyomata of the heart is the association with cerebral sclerosis. An inspection of the following table will show that of the twelve cases assembled seven had cerebral sclerosis. Of the remaining five cases no mention of the head was made in three, and in two cases, Seiffert's and my own, it is known that the cortex was normal. Ponfick and Bonome are the only two observers who have dwelt upon this association. Ponfick merely emphasizes this fact. Bonome has sought an explanation and a possible common factor in their causation in the evidences of fetal malnutrition with secondary vascular degenerations. This hypothesis has much in its favor. Virchow in a consideration of encephalitis congenita states that the lesions are due to vascular changes dependent upon malnutrition and has demonstrated fat in the vessel walls.

* I am indebted to Professor F. B. Mallory for this observation.

Case.	Age.	Number of Tumors.	Cerebral Sclerosis.	Remarks.
Von Recklinghausen	New born.	Multiple.	Present.	
Virchow.....	"	"	Head not mentioned.	Well nourished.
Hlava	14 days.	Single.	" " "	
Kolisko.....	2 months.	Multiple.	Absent.	
Cesaris-Demel	3 years.	"	Present.	
Seiffert	1 yr. 8 mos.	"	Absent.	Rachitis.
Rothe	?	"	Present.	
Ponfick, Case I.	7 months.	"	"	No anatomical peculiarities.
Ponfick, Case II.	3 years.	"	"	Chronic furunculosis; emaciation.
Bonome	1 yr. 6 mos.	"	"	Rachitis.
Knox and Shorer....	7 months.	"	Head not mentioned.	"
Wolbach	10 months.	Single.	Absent.	Scorbutus, rachitis, spina bifida, neuroglia nests in meninges of spinal cord.

Inspection of the table shows the number of cases associated with disturbances of nutrition. It is unfortunate that a number of the descriptions of rhabdomyomata do not include a statement of the general anatomical conditions. Bonome lays stress upon the association of cerebral sclerosis with nutritional diseases. Yacobaeus¹⁶ has collected twenty-five cases of "hypertrophic tuberous cerebral sclerosis," seven of which were associated with multiple tumors of the kidneys. He states that a histological examination of the kidney tumors in most cases was not made. A few cases have been smooth muscle tumors and renal adenomata. Yacobaeus' case was a leiomyoma with peculiar arrangements in relation to blood vessels and hence called by him an angio-myo-sarcoma.

The association of congenital rhabdomyomata with cerebral sclerosis and nutritional diseases on the one hand and the association of cerebral sclerosis with nutritional diseases and multiple tumors on the other hand are now well-established facts. The significance of these facts and their bearing upon general problems are beyond the scope of this

paper. The occurrence of multiple neuroglia nests in the meninges of the cord in my case I believe should be classed as a developmental anomaly, and accordingly unlike in nature to the diffuse cerebral sclerosis associated with other cases.

SUMMARY.

There are now twelve authentic cases of congenital rhabdomyomata of the heart, six of which were associated with diffuse cerebral sclerosis. In three cases the head was not examined so that the percentage of this combination is possibly higher. Most of these cases have been associated with general disturbances in nutrition.

In the case here reported there was found a condition undescribed in pathological literature, the multiple nests of neuroglia tissue in the meninges of the spinal cord.

The spongy or vacuolated structure of the rhabdomyomata of the heart is due to spaces within the tumor cells.

The fuchsinstaining granules which may be irregularly distributed in the tumor cells or arranged in an orderly fashion to form cross striations represent sarcous elements.

The longitudinal striations are made up of alternating sarcous elements and fibrillary material, which stain respectively red and blue with Mallory's connective tissue stain.

The tumor cells may produce either an excess of sarcous elements or of fibrillary material.

The presence of non-striated fibrils in tumor cells may be explained by an excessive production of fibrillary material.

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DESCRIPTION OF PLATES.

PLATE XXV.

FIG. 1. — Heart with right ventricle exposed. Slightly less than natural size.

FIG. 2. — Low power view of section through base of tumor. Shows the sharply defined boundaries of the tumor and the spongy structure. Phosphotungstic acid hematein stain.

FIG. 3. — A single cell with processes and a space wall of an adjacent cell showing cross striations. Phosphotungstic acid hematein stain. x 1,000.

PLATE XXVI.

FIG. 4. — A single cell with parallel fibrillæ. This is the nearest approach to normal muscle found in the tumor. The striations are composed of regularly arranged dots along the courses of the fibrillæ. Note isolated fibrils with dots. Phosphotungstic acid hematein stain. x 1,000.

FIG. 5. — A spider cell, first described by Cesaris-Demel. The nucleus lies in a mass of protoplasm attached to the cell wall. In places striated

fibrillæ can be seen in the cell walls. The processes from the central mass of protoplasm contain striated fibrillæ and join the cell walls. Phosphotungstic acid hematein stain. x 1,000.

PLATE XXVII.

FIG. 6. — Two cells, one of them containing a large cavity. Selected to show the intracellular location of the tumor spaces. Phosphotungstic acid hematein stain. x 1,000.

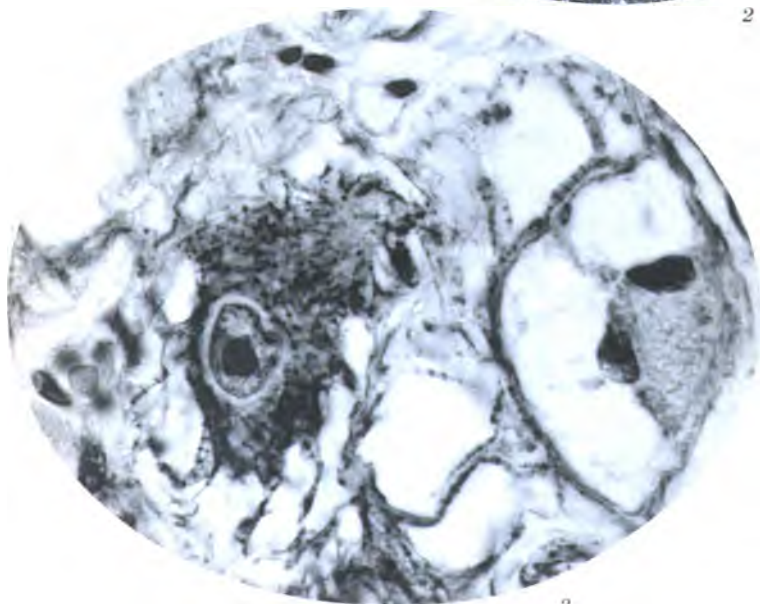
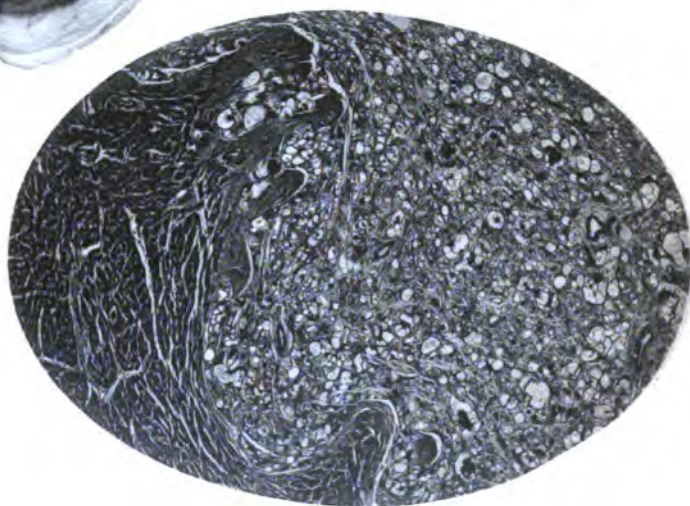
FIG. 7. — A single cell with processes showing details of fibrillæ and irregular arrangement of the primitive sarcois elements in central portion of the protoplasm. Phosphotungstic acid hematein stain. x 1,000.

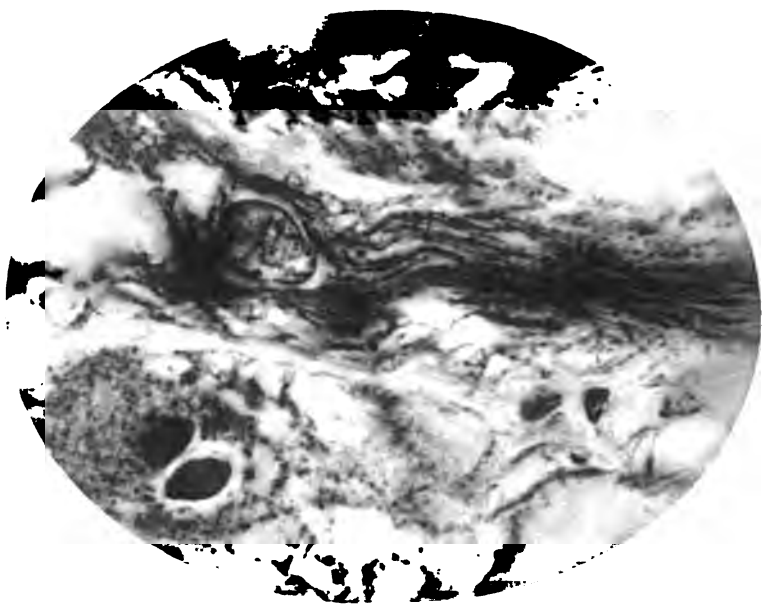
PLATE XXVIII.

FIGS. 8 and 9. — Details of a cell similar to that of Fig. 7. Show large numbers of sarcois elements for the most part irregularly arranged. A few are connected by delicate fibrils. Phosphotungstic acid hematein stain. x 2,000.

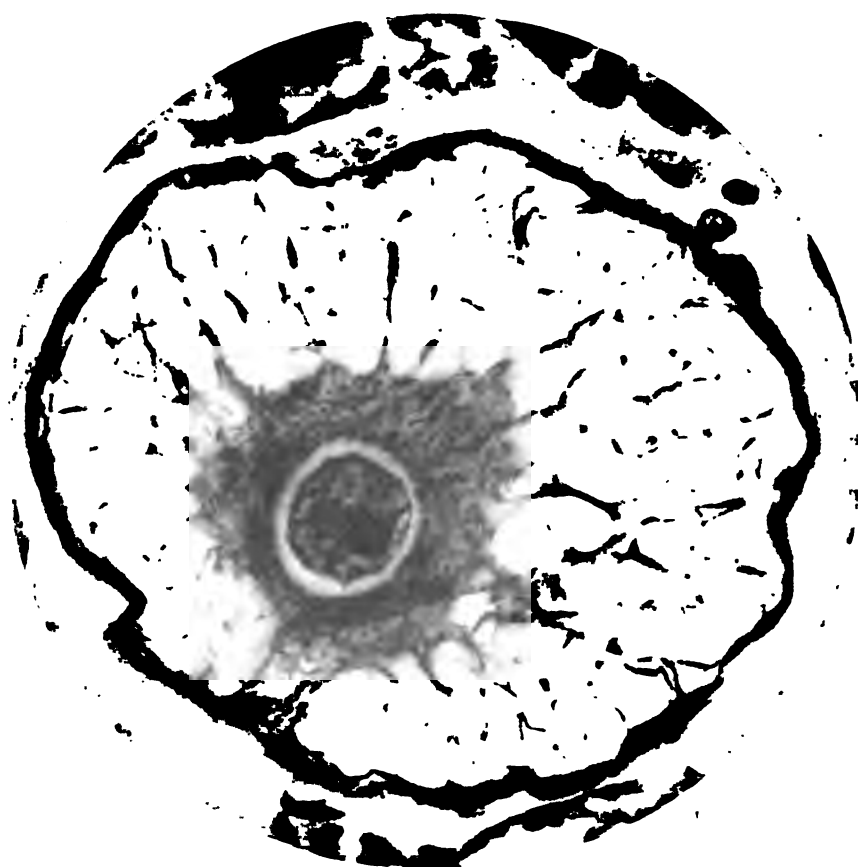
FIG. 10. — A single cell with three nuclei and processes containing non-striated fibrils. Phosphotungstic acid hematein stain. x 500.

FIG. 11. — Edge of spinal cord with pia arachnoid showing neuroglia nests. Phosphotungstic acid hematein stain.

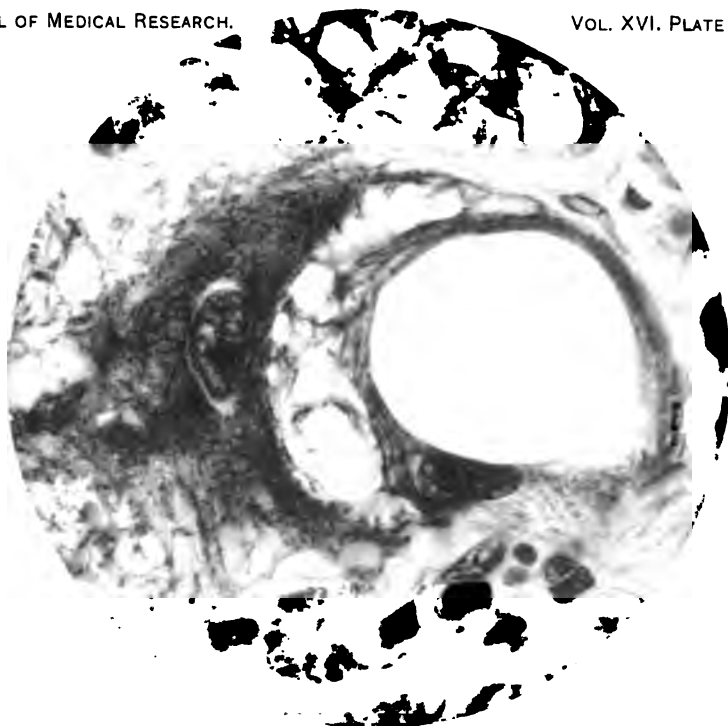




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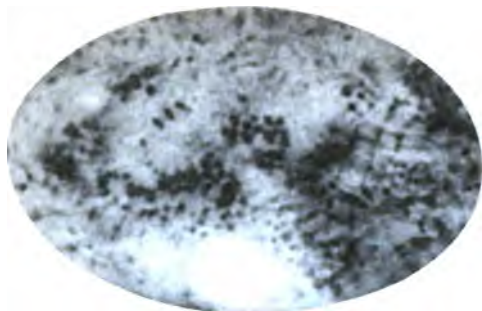
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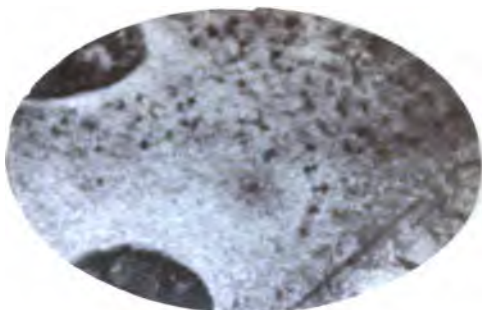
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Wolbach.

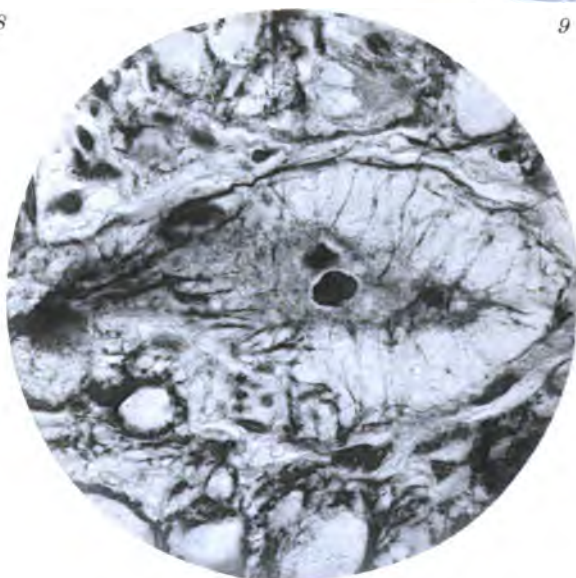
Rhabdomyom



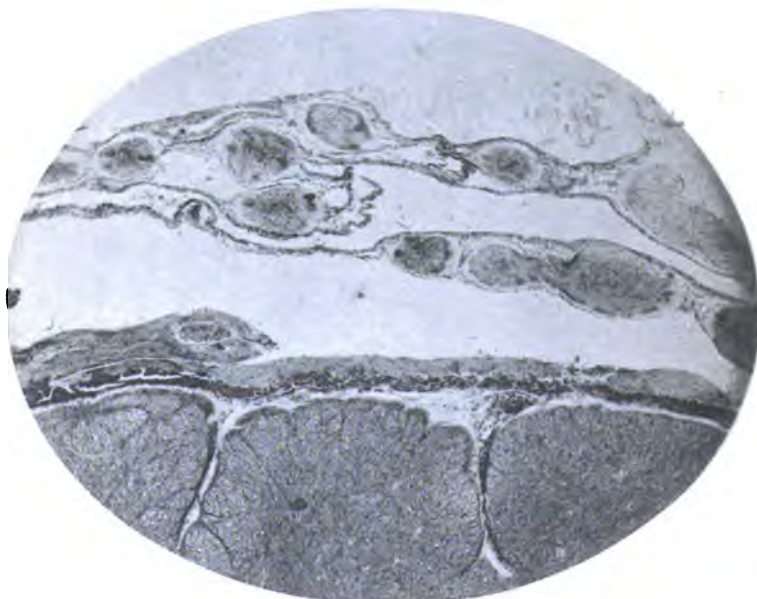
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11



OBSERVATIONS ON PHAGOCYTOSIS IN RELATION TO THE
OPSONIC INDEX.*

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While the phenomenon of phagocytosis of bacteria has been known and studied for a considerable time, it is only within the past few years that some of the causes and the laws governing the action have become better understood.

The best known cause of phagocytosis at present, and the one occupying the attention of medical men almost exclusively, is the opsonin of the blood serum, first clearly demonstrated by Wright and Douglas.

Of the several protective bodies known to exist in normal and immune sera, only the opsonins can be quantitatively determined with any considerable degree of accuracy by any methods so far discovered.

It should be remembered, however, that all the immune protective bodies arise from the action of the bacteria and their chemical products; so that, while the opsonins are distinct from the others, the probable quantity of the others may at least be inferred from the amount of opsonin found to be present.

In order that phagocytosis may indicate the relative quantities of an opsonin in two sera, special technical methods must be employed and certain principles followed. Lack of knowledge of the principles governing phagocytosis causes more inconsistent and erroneous opsonic findings than failure to follow the technical rules so frequently published by various workers.

It is the purpose of this paper to describe some experiments which may throw some light on such errors.

Staphylococci of various strains were used in all the experiments.

*Read before the American Association of Pathologists and Bacteriologists at Washington, D.C., May 8, 1907. Received for publication June 3, 1907.

In the method of technic used by those who follow Wright, and this includes all workers excepting those who use Simons' method, equal proportions of pure blood serum, washed leucocytes and a suspension of staphylococcus in normal salt are intimately mixed and incubated for a definite time — usually fifteen to thirty minutes.

Now every one knows how difficult it is to maintain the virulence of a strain of staphylococcus; and it must be common knowledge to all workers with opsonins that the opsonin normally present in blood serum is sufficient to sensitize such a large number of ordinarily virulent staphylococci that in the usual Wright preparation the phagocytosis would be far too great for accurate or convenient counting.

In order to reduce the phagocytic index to an average that can be readily and quickly counted, the method generally employed is to dilute the bacterial suspension to the degree required, as shown by experience.

I shall try to show that such a method will not and cannot give any true indication of the relative opsonic value of the sera tested.

Essential processes in phagocytosis are:

1. The sensitization of the bacteria, and
2. The phagocytosis of the bacteria so sensitized and only those.

The first process, of course, must precede the second.

The rate of sensitization will be, presumably, proportional to the quantities of opsonin in the sera used. If this rate be slow, extending over the incubation interval of fifteen to thirty minutes, then, even with a thin suspension of bacteria, we might expect phagocytic indices proportional to the opsonin in the sera. On the other hand, if sensitization be a rapid process, completed within the first few moments of the incubation period, leaving a considerable interval without fresh addition of sensitized bacteria, phagocytosis following quickly on preparation should, if all the bacteria introduced are sensitized by each serum, give equal indices. If too few bacteria are present to satisfy all the opsonin of the stronger serum, the weaker one being satisfied, errors of all degrees should be possible.

It is then important to know something of the time needed for the completion of the reaction of sensitization. To investigate this, a mixture of dense staphylococcus suspension in normal salt with an equal quantity of a normal serum (both at 37° C.) was made and incubated. At intervals of one, three, five, ten, fifteen, and twenty minutes after making the mixture, double portions of it were withdrawn, mixed with single portions of washed leucocytes, and incubated for thirty minutes. The results are shown in Table I.

TABLE I.

Intervals after Mixing Serum and Bacteria.	1.	3.	5.	10.	15.	20.
Average phagocytosis	10.30	10.28	10.38	10.14	10.15	10.35

It appears from this experiment that the process of sensitization is very early completed. It follows, then, that if the bacterial suspension contains fewer germs than the opsonin of either serum is able to sensitize, the result should be that each will sensitize all the bacteria present, and equal phagocytic indices will be observed. In such a case the opsonic index obtained will be unity regardless of the real difference between the sera. If all the opsonin of the weaker serum find bacterial attachment, but too few bacteria are introduced to combine with all the opsonin in the stronger serum, an error depending on the quantity of opsonin not combined will result.

That these deductions are true is shown by the following experiment:

Seven bacterial suspensions having their densities in the proportion of one, two, four, eight, sixteen, twenty-four, and thirty-two were prepared. Two sera, one twice the strength of the other, were made by appropriately diluting a serum with normal salt solution.

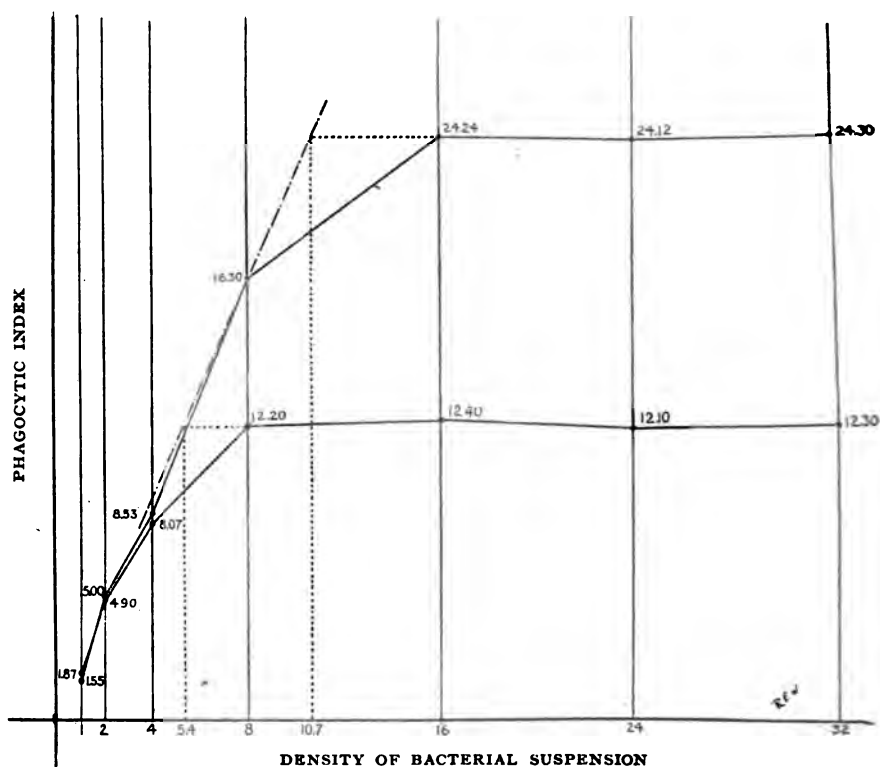
Using the weaker serum as a normal, the opsonic index of the stronger was obtained by using each of the seven bacterial suspensions.

The results are shown in Table II.:

TABLE II.

Relative Density of Bacterial Suspension.	1.	2.	4.	8.	16.	24.	32.
Phagocytic Index for Serum No. I.....	1.87	4.90	8.07	12.20	12.40	12.10	12.30
Phagocytic Index for Serum No. II.....	1.55	5.00	8.53	18.30	24.24	24.12	24.30
Opsonic Index for Serum No. II.....	.83	1.02	1.05	1.5	1.95	1.99	1.98

These results plotted as curves, having the density of bacterial suspension and the phagocytic index as ordinates and abscissæ, show some very interesting points.



For each serum the phagocytic index advances nearly in the same proportion as the numbers of bacteria used, up to a certain point. For the weaker serum this proportion is lost after the third suspension, the index for the fourth being only 12.20 instead of about sixteen. This means that the serum could sensitize only enough bacteria of those present in the fourth suspension to produce under the circumstances of the experiment a phagocytosis of 12.20. Two, three, and four times the number of bacteria used in the fourth suspension gave practically the same phagocytic index for the serum. Under the circumstances of the experiment the weaker serum was able to produce a maximum phagocytic index of 12.20. Excess of bacteria, above what were needed for this result, had no effect because the excess could not be sensitized by the serum and only sensitized bacteria are taken up.

By extending the general line of the curve for the first three points, until it intersects the phagocytic line of 12.20, we obtain as the density of bacterial suspension just sufficient to satisfy all the opsonin in the weaker serum, that density which corresponds to 5.4 times the thinnest suspension used.

Examining the curve for the stronger serum in the same manner, it is seen that its maximum phagocytic index is not reached until the fifth bacterial suspension was used. Double and treble the number of bacteria in the suspension did not increase this maximum index of 24.24.

The density of bacterial suspension just sufficient to produce an index of 24.24 for the stronger serum is found to correspond to 10.7 times the density of the thinnest suspension used in the experiment; and this is just about double the corresponding suspension for the weaker serum, viz.: 5.4.

It is apparent that any density of bacterial suspension less than 10.7 times the minimum suspension used must yield an erroneous opsonic index; and that this error may vary anywhere between 1 and the true index of 2.

In the experiments just described the two sera corresponded closely to normal serum diluted fifteen and thirty times respectively.

The thinnest bacterial suspension contained about 1,000,000 cocci per cubic millimeter.

Undiluted serum would require about fifteen times as dense a suspension as the one just sufficient to produce the maximum phagocytic index of 24.24 in the curve, and would allow an average of 363.6 cocci per leucocyte, supposing that the cell could engulf that many.

It must be clear, then, that unless one has a strain of staphylococcus of exceptional virulence the use of undiluted serum is impracticable.

A method of technic based on the work described, and which has given good results, is to dilute the sera ten to twenty-five times in one of the tubes described by Wright for making higher dilution at a single step, and to use the diluted serum with a very opaque suspension of staphylococci.

It seems scarcely necessary to add that the principle of using enough bacteria to produce the maximum phagocytosis of which the serum is capable applies to all bacteria which are rapidly sensitized.

However, for species for which but little opsonin exists in the blood, as for example the typhoid bacillus, comparatively fewer germs are necessary and the serum need not be diluted.

No harm will follow the use of thick bacterial suspensions in such work and errors may thus be avoided.

[In concluding this paper I desire to express my thanks to Dr. George Dock and the members of his hospital staff for the encouragement and valuable suggestions and criticism, without which my observations could scarcely have been carried out.]

A NEW PATHOGENIC MICROÖRGANISM OF THE CON-
JUNCTIVAL SAC.*

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During the last few years the widening of bacteriological methods in ophthalmology has been extensive. In 1883 Koch, while working in Egypt, examined the conjunctival discharge in some cases of Egyptian ophthalmia. Among the catarrhal forms he found constantly a very small fine bacillus, similar in size to the bacillus of mouse septicemia. In 1887 Weeks reported having seen epidemics of conjunctivitis in New York, in the conjunctival discharge of which he constantly found a very small fine bacillus. This was the same microörganism which Koch had seen in Egypt. The cultivation of this bacillus was extremely difficult. Weeks was at first only able to cultivate it along with *Bacillus Xerosis*, while Koch's attempts at cultivation had been unsuccessful. The organism and the conjunctivitis were called Koch-Weeks. Since described, Koch-Weeks conjunctivitis has been studied extensively, the clinical characteristics of the disease and the cultural features of the bacillus established. Its presence has been reported from many different countries and it is to-day recognized as one of the most contagious and best known diseases of the conjunctival sac.

Neisser in 1879 had described the gonococcus as seen in the pus of gonorrheal affections of the eye, but it was not until after the Koch-Weeks discovery that much activity was shown in studying the bacteriological factors in conjunctivitis.

In 1896 Morax and Axenfeld described a form of conjunctivitis with definite clinical features which was caused by a diplo-bacillus. This has since been called Morax-Axenfeld Conjunctivitis. It is to-day one of the most frequently seen diseases of the conjunctiva.

* Received for publication June 5, 1907.

In 1893 Gasparini reported the pneumococcus as a cause of conjunctivitis and keratitis, whilst later a form of conjunctivitis which usually accompanied systemic influenza was attributed to *Bacillus influenzae*.

The work thus begun by Koch and Weeks and further pursued by Morax, Axenfeld, Uhthoff and others has borne such fruit that to-day we are able to divide the different forms of conjunctivitis not so much from their clinical characteristics as from the bacteriological findings.

During the last two years we have been doing this bacteriological work at the Montreal General Hospital, to which institution upon April the sixth, 1907, a mother brought her infant of nine months and complained that the baby's eyes had been "sore and running" for five days. The clinical picture was as follows: both eyes were involved, upon the lashes was much dried secretion. The palpebral conjunctiva was intensely congested, while the bulbar conjunctiva was quite normal. The lids were not red or swollen. From the conjunctival sac there was a profuse muco-purulent discharge freely mixed with tears.

A little of the discharge was smeared well over a glass slide, fixed, and stained by Gram's method, using as a counter-stain a weak solution of safranin. Upon examining the slide I saw here and there tiny Gram negative bacilli which seemed too short and thick for the Koch-Weeks bacillus and too thick for *Bacillus influenzae*. This same bacillus I had seen before but in the hurry of a large outdoor clinic I had not pursued it farther, contenting myself with the thought that it was *Bacillus influenzae*.

Tubes of blood serum, plain agar, bouillon, and hemoglobin agar were then inoculated and put in the incubator. After twenty-four hours a growth was seen upon the hemoglobin agar. The other tubes were negative. Smears showed this same small Gram negative bacillus with staphylococci. The bacillus, after a few days, was obtained in pure culture. A conjunctival sac was now inoculated, a conjunctivitis set up, and, from the conjunctival discharge, the same small bacillus was obtained.

Within a month nine such cases have presented themselves at the out-door department. They have all been infants, and all from among the Jewish colony here. They have all shown definite characteristic clinical features and from each case this same bacillus has been isolated.

Smear preparations. — In making a smear a loopful of the conjunctival discharge should be teased well over a glass slide, fixed, and stained preferably by Gram's stain, using as a counter-stain a five per cent watery solution of safranin. Gram's stain is especially useful in conjunctival work eliminating immediately the frequently present Gram positive bacilli. Upon examining the smear, one finds here and there tiny bacilli. They are short and thick, their rounded ends giving the appearance of elongated cocci. They may be found in groups but seem to lie preferably singly. They will be found both within and without the leucocytes, generally without.

From culture the bacillus seems to be slightly larger and tends to appear as diplo-bacilli. It is short and thick with very pointed ends, is $.5-2.0\mu$ long by $.3-0.4\mu$ wide. The bacillus shows uniform staining, has no capsule, is non-motile and does not form spores. It is decolorized by Gram's stain, but stains well with weak solutions of carbol fuchsin or methylene blue.

Cultural features. — The tubes of hemoglobin agar inoculated with some of the conjunctival discharge gave a pretty and characteristic result which was constant in all the cases. After twenty-four hours in the incubator one sees over the surface numerous separate colonies of *Staphylococcus albus*. Around each of these separate colonies radiating out from them, as centers, are seen very fine, colorless, shiny rounded colonies. These colonies are exceedingly fine, seem to keep separate, and extend over the whole surface. The original cultures from all the cases were as the one described and showed how well this bacillus grows in commensal relationship with the staphylococcus.

From the original tube the bacillus was obtained in pure culture by surface seeding a plate of hemoglobin agar. In trying to obtain it in pure culture by transferring from tube to tube, the growths were always contaminated by the staphylococcus. Over a plate of hemoglobin agar, however, by smearing a loop inoculated from one of the small colonies as far from the albus as possible the bacillus was readily obtained (in pure culture).

The growth of the bacillus on hemoglobin agar is characteristic. After twenty-two to twenty-four hours in the incubator the slant will be seen covered with a mass of tiny colonies. They remain separate and distinct, are colorless, and as will be seen from the tubes the colonies are no larger than the sharp end of a pin. Indeed the growth though profuse is at times exceedingly difficult to recognize. It is seen much better by artificial than by daylight, especially with the proper reflection of light. The growth of the bacillus is very easily affected even upon hemoglobin agar, upon which it grows best. Upon freshly made hemoglobin agar it will not grow in less than forty-eight hours. The reaction of the media, too, is of the greatest importance. Hemoglobin agar, neutral, 1.0 alkaline, .65 acid, 1.0 acid, and 1.75 acid to phenolphthalein was made up. Upon alkaline media it would not grow. Upon the neutral and .65 it was with great difficulty transferable. The most satisfactory reaction, and the one which has been latterly used, is the 1.0 and 1.75 acid.

The cultivation of this bacillus on media, upon which it seemed to thrive best, has been at times unsatisfactory and extremely difficult. Upon the same batch of hemoglobin agar sometimes it will take forty-eight hours before there is any sign of a growth. At other times cultivation is comparatively easy.

Upon glycerine agar after twenty-four hours in the incubator a growth is seen. The surface of the agar is covered with these same tiny pin-point colorless colonies. They are here hardly perceptible but a smear from the surface will show their presence in quantity.

Upon hydrocele agar the growth is exceedingly fine and is only perceptible with the proper reflection of light. From behind no growth will be seen but smears from the surface will show the presence of the organism. Upon plain agar the appearance of the growth is similar to that upon hydrocele agar. The growth upon plain agar is by no means constant. Upon blood serum the results have been negative. In bouillon there has never been any growth, indeed, even in the water of condensation from a slant where there is a profuse growth, the bacilli are very few in number. To tubes of litmus agar were added dextrose, dextrin, maltose, lactose, saccharose, galactose, innulin, mannit. The growths here were sparse. Over the surface of the agar were seen these same tiny colorless pin-point colonies. To similar litmus agar tubes to which the sugars had already been added were added a few drops of blood. The growth here was profuse with no change in the reaction. The bacillus has been cultivated, too, upon Dorset egg medium. The appearance of the growth is as if the surface of the medium had been slightly roughened with a swab stick.

To sum up its cultural characteristics, this bacillus grows only at the body temperature and is aërobic. It grows best upon hemoglobin agar of 1.0 and 1.75 acid reaction. The growth upon hemoglobin agar is very easily affected and the cultivation of the bacillus is at times difficult. In commensal relationship with staphylococcus albus the bacillus grows well.

It grows on Dorset egg medium, glycerine and hydrocele agar and has been cultivated upon plain agar. It does not grow in bouillon or in any liquid medium or upon blood serum. It does not produce gas, does not ferment any sugars, and has no chromogenic characters.

Viability.—The cultures on hemoglobin agar remain viable from two to three weeks provided there is sufficient water of condensation in the tubes. One culture inoculated on April eleventh was transferable April twenty-fifth, but not on May third. It had not been kept in the cold nor in any way taken especial care of.

Pathogenicity. — For the human conjunctival sac this bacillus is pathogenic. A conjunctival sac which was found to be free from microorganisms was flushed well out with warm water. Fifteen minutes later the sac was inoculated with a loopful of a twenty-four-hour growth. Twelve hours later there was a feeling of "something in the eye" and six hours later the eye showed a marked catarrhal conjunctivitis. The palpebral conjunctiva of the lower lid was especially involved. The posterior conjunctival vessels were somewhat dilated, otherwise the bulbar conjunctiva was not involved. From the muco-purulent discharge, which was here, too, freely mixed with tears, the organism was obtained.

With the washing from a slant on hemoglobin agar an ordinary house mouse was inoculated intraperitoneally. Eighteen hours later the mouse was unable to move, and six hours later was dead. At autopsy the peritoneum was found injected and filled with a gelatinous sticky exudate. Smears showed the pus cells filled with the bacilli, with little or no fibrin. Inoculations from the peritoneum on hemoglobin agar gave pure growths of the bacillus.

This tube was washed down and inoculated intraperitoneally into a white mouse. Twenty hours later the mouse was dead. Post-mortem was found marked peritonitis of the gelatinous type similar to Case I. From the peritoneum here was obtained the bacillus in pure culture. A different strain of the bacillus on hemoglobin agar was now taken and the wash similarly injected into a white mouse. Sixteen hours later the mouse seemed very ill, was unable to stand up or move, and four hours later was dead. From the peritoneum, which showed a marked peritonitis similar to the previous cases, two tubes of hemoglobin agar were inoculated which twenty-four hours later gave a pure growth of the bacillus. Hemoglobin agar was also inoculated with the heart's blood, which gave twenty-four hours later a pure growth of this same bacillus. Two house mice were then inoculated with two different strains; twenty-four hours later they were both dead. Post-mortem the marked gelatinous peritonitis was found from which pure cultures were obtained, also

pure cultures from the heart's blood. In one case two tiny loopsful from the heart's blood gave a growth covering the entire surface of the agar. To check these experiments, on April twenty-ninth last, four mice (two white and two house mice) were inoculated intraperitoneally with four different strains of the bacillus. The inoculations were done at four P.M. April twenty-ninth. At noon the following day one white mouse and one brown were dead. The other two were unable to move or stand. Post-mortem the peritoneum in all cases was found involved as before. From the peritoneum and from the heart's blood in each case were obtained pure growths.

Discussion and differentiation.— This microörganism, then, a cause of conjunctivitis, needs to be differentiated from the different known bacteriological causes of conjunctivitis. Differentiation from Morax-Axenfeld's diplo-bacillus, Petit's diplo-bacillus, Friedländer's bacillus, *Bacillus coli*, and *Bacillus subtilis* may be dealt with in a few words. It is far too different morphologically and culturally to necessitate going into minutiae here. The pathogenic microörganisms of the conjunctival sac which it must be differentiated from, however, are the Koch-Weeks bacillus and the different influenza bacilli; namely, the influenza bacillus of Pfeiffer, the so-called influenza bacillus of conjunctivitis of Müller, and the pseudo-influenza bacillus of Zur Nedden.

1. Clinical considerations: While the different bacteriological factors causing conjunctivitis do not always, yet, in the majority of cases these organisms do, give rise to well marked characteristic clinical pictures. In Koch-Weeks conjunctivitis one finds reddened and edematous lids, intense injection of the bulbar conjunctiva, with profuse muco-purulent discharge. In influenza conjunctivitis there is a well marked involvement of the bulbar conjunctiva and signs of influenza elsewhere. The *Bacillus influenzae* has been found in the normal conjunctival sac and in odd cases of conjunctivitis where there were no general signs of influenza, but in the majority of cases influenza conjunctivitis accompanies

systemic influenza. Clinically, Koch-Weeks, influenza, and the form of conjunctivitis here described are very different.

2. Morphology: Differentiation by morphology is unsatisfactory and here unnecessary. While the Koch-Weeks bacillus may be differentiated from the bacillus influenza by its appearance on the slide, and while the bacillus here described seems shorter and thicker than the bacillus influenza, I am ready to admit that morphologically they are strikingly similar.

3. On media these organisms differ widely. The Koch-Weeks bacillus does not grow well on hemoglobin agar, as here used. It grows best on hydrocele or ascitic agar, where even growing with the bacillus xerosis it is hardly perceptible and dies out very quickly in forty-eight hours or a little longer.

The *Bacillus influenzae* in the presence of hemoglobin after twenty-four hours shows a profuse raised growth of round whitish colonies. The growth is easily seen, the colonies being the size of an ordinary pin head. Older colonies show some change in color.

The bacillus here described, on hemoglobin agar, gives a very characteristic appearance. Over the surface of the agar, with a proper reflection of light, is seen a mass of tiny pin-point colorless colonies. The growth is not easily seen. This organism has also been cultivated on media free from hemoglobin.

4. Pathogenicity: The Koch-Weeks bacillus while pathogenic for the human conjunctival sac is not pathogenic for animals. The *Bacillus influenzae* is slightly pathogenic for some animals. Pfeiffer found by injecting the *Bacillus influenzae* intravenously into rabbits a characteristic effect was produced. He attributed these results to toxic products present in the cultures and in none of his experiments was he ever able to obtain effects resembling septicemic infection. Cantani by injecting the bacillus influenza into the anterior portion of the brain of rabbits succeeded in producing an acute encephalitis. The bacilli, however, were never found in the blood or other organs.

This bacillus is pathogenic for mice. In every case where

injected intraperitoneally a marked peritonitis was set up, and a septicemia, as evidenced by pure cultures of the bacillus, being obtained from the heart's blood.

This organism, then, is similar to the different influenza bacilli in morphology and in its growing well in commensal relationship with staphylococci.

It is not *Bacillus influenzae* for the following reasons:

(1.) The form of conjunctivitis set up differs from influenza conjunctivitis.

(2.) This bacillus on hemoglobin agar differs widely from *Bacillus influenzae*. On this media under most favorable conditions and with the media at a most suitable reaction the growth is hardly perceptible, and differs from *Bacillus influenzae* on the same media in size, color, and appearance.

(3.) The bacillus has been cultivated in media free from hemoglobin.

(4.) It is viable for a much longer period than *Bacillus influenzae*.

(5.) It is pathogenic for mice, causing a septicemia.

CONCLUSIONS.

We have, then, here to deal with a new form of conjunctivitis set up by a new etiological factor. That factor resembles somewhat the bacillus of influenza, but differs from it widely on media, viability, and pathogenicity. From the examination of five hundred cases of conjunctivitis during the last year and a half this series of cases, occurring within a month, seems to be different from previously described types. Nine cases, I recognize, is a small number to draw conclusions from, but the characteristic clinical features of these cases caused by an organism not as yet described as a pathological factor in conjunctivitis leads me to the conclusion that we have here to deal with a new form of conjunctivitis set up by a new organism of the conjunctival sac.

Until the clinical features from which the bacillus should take its name are better understood, I shall call it *Bacillus McKee*.

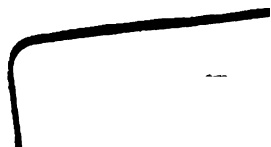
[I take great pleasure in expressing my thanks to Dr. C. W. Duval, the director of the laboratory, for much assistance while doing this work.]

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